PLATES

PLATE I.

Rock, gravel and organisms dredged from Okhotsuk Yamato Bank.

Fig. 1. Rock fragment dredged from 44° 45.4' N. 144° 20.8' E. 185m.
Left side: *Stylaster* sp. attached on carcareous shale
Center: *Hyodrozoa* attached on sandy shale.
Right side: *Bryozoa* attached on conglomerate.

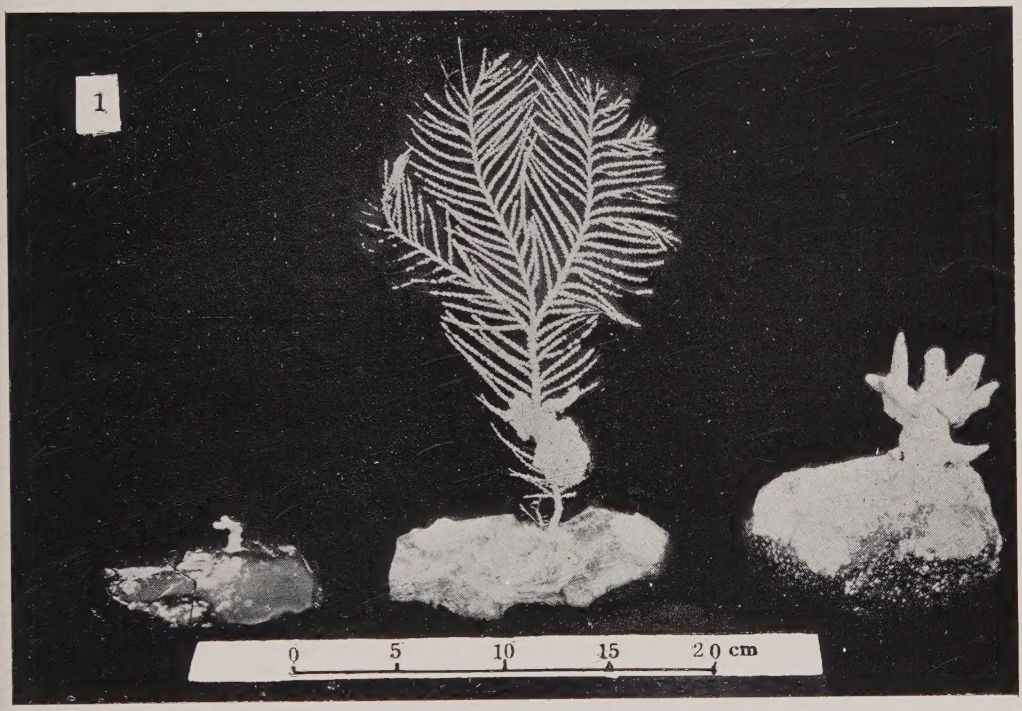
Fig. 2. A fragment of marly shale. Same station to Fig. 1.

Fig. 3. Gravel of greywacke sandstme. organisms attached on the surface. Same station Fig. 1.

Fig. 4. Gravel cemented togather by sponge. Same station Fig. 1.

Fig. 5. *Flavellum* sp. (cfr. *transversale conicum* Yabe et Eguchi) Same station Fig. 1.

Fig. 6. 7. *Limposis* sp. Same station Fig. 1.


1
0 5 10 15 20 cm

4
2
5
0 1 2 3 4 cm
0
1 cm
2
6
3
7

PLATE II.

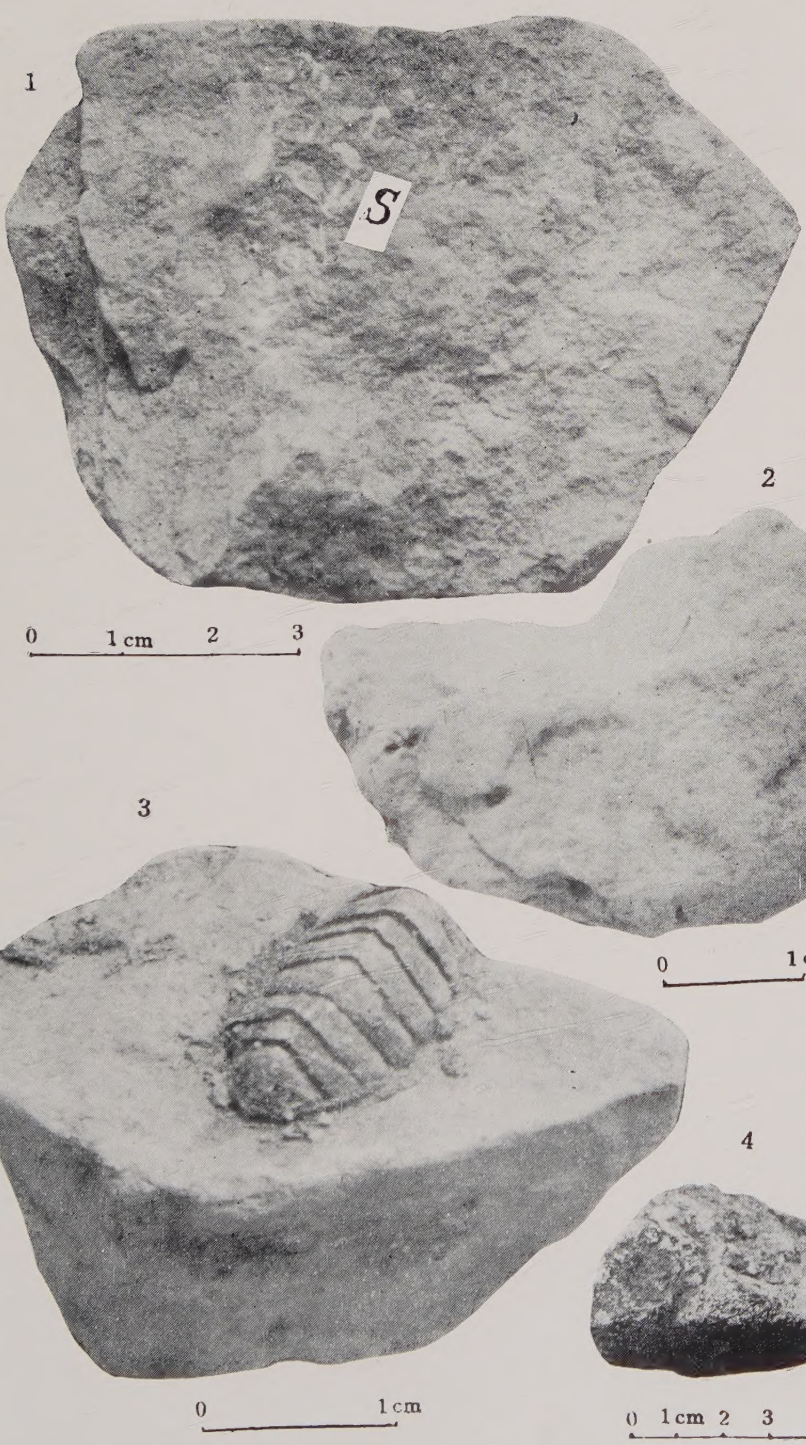
Rock and gravel dredged from newly discovered bank.

Fig. 1. Diatomaceous shale dredged from Erimo Bank, St. 1 41° 36' N. 143°38' E. 150 m.
S: fossil sponge *Sagarites chitanii* Makiyama contained in the rock. This fossil is commonly found in the Neogene Tertiary in Japan.

Fig. 2. Diatom earth from Mogami Bank, Japan Sea.
39° 10.8' N. 138° 51.8, E. 240 m.

Fig. 3. Fragment of sandy shale from Erimo Bank.
41° 38.3' N. 143° 37.6' E. 170 m.
Lepridozona sp. attached on the surface.

Fig. 4. Gravel dredged from Eriono Bank. Station: Same station to Fig. 3.


1
S
0 1 cm 2 3
2
0 1 cm
3
0 1 cm
4
0 1 cm 2 3 4

LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS
OCT 28 1953

JOURNAL

OF THE

TOKYO UNIVERSITY OF FISHERIES

VOL. XXXIX, NO. 1.

CONTENTS

ONO, T. and NAGAYAMA, F.: Biochemical studies on the vitamin A in fish viscera. 2. Relation between the autolysis of liver and the vitamin A potency of oil 1– 14

OKADA, I., OSAKABE, I., KURATOMI, Y. and KIKUCHI, T.: Dehydration of the Agar-agar-gel. Part 3. Dehydration by pressure.. 15– 30

OKADA, I., OSAKABE, I., KURATOMI, Y. and KIKUCHI, T.: On the studies of glutathion in the marine products. Part 1. Polarographic determination of glutathion in seaweeds 31– 38

OKADA, I., HONDA, K., TOYAMA, K. and KIKUCHI, T.: On the efficacy of net preservatives. Part 1. About some aromatic chlorine derivatives..... 39– 46

YOSHIHARA, T.: Effect of population-density and pond-area for the growth of fish..... 47– 61

YOSHIHARA, T.: A table and alinement chart to facilitate the calculation of the condition factor..... 63– 67

HOSHINA, T.: Notes on some myxosporidian parasites on fish of Japan..... 69– 89

KUBO, I.: On two rare species of Psalidopodidae and Nephropsidae. 91–100

NIINO, H.: On the bottom character of the insular shelf around Hachijo Island and the neighbouring Banks..... 101–110

THE TOKYO UNIVERSITY OF FISHERIES
KURIHAMA, YOKOSUKA CITY, KANAGAWA PREF., JAPAN
December, 1952.

JOURNAL OF THE TOKYO UNIVERSITY OF FISHERIES

Editors for this Journal

Ikunosuke Okada, Professor, Dr.

Hiroshi Niino, Professor, Dr.

Itsuo Kubo, Professor, Dr.

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

BIOCHEMICAL STUDIES ON THE VITAMIN A IN FISH VISCERA
II. RELATIONS BETWEEN THE AUTOLYSIS OF LIVER AND THE VITAMIN A POTENCY OF OIL

Toyoki Ono and Fumio Nagayama

(Received on Sept. 15, 1952)

For the extraction of vitamin A, D from fish liver, it is commonly used the manufacturing method, based on alkali digestion of liver, from which some stable liver oil for oxidation is produced. The storage of fish liver for a long time, or at a higher temperature, lead to the loss of vitamin A potency. We suppose it will be resulted from the decomposition of antioxidants in liver in addition to the oxidation of vitamin A or from the activation of an unknown enzyme oxidizing vitamin A. The hypothesis have been proved by the following experiment.

Storing at room temperature the liver oils of great blue shark (Yoshikirizame), produced by the alkali digestion method and the autolysis process, the loss of vitamin A increased in the latter. Since the ratio of both losses was one to ten. We suppose that the liver oil obtained by autolysis may have the lower content of antioxidants than the oil by other processes. These figures have been ascertained again with the experiments using skipjack and mackerel livers.

The mackerel liver oil, obtained by the autolysis for 5 hours at 60°C, did not almost contain vitamin A and changed to a completely oxidized product. On the contrary, the liver oil extracted through the alkali digestion process was not occured any oxidation.

The destruction of vitamin A developed during the autolysis of mackerel liver is not only carried on the oxidation but may be induced by a special enzyme action such as the oxydase for vitamin A.

Sumner[1], Strain[2] and Ball[3] found the carotene-destroying enzyme to be identical with lipoxidase and strated on the occurence, action, specificity and activation. Lipoxidase occures in various plant; in fatty and muscular tissue of pigs[4]; in herring muscle[5]; in rat mucosa[6]. Theorell[7] has isolated it in a pure crystaline state. However, in regard to the mechanism of oxidation on the vitamin A or unsaturated fatty acid by the lipoxidase, the experimental result has not been satisfactory. We are now making some progresses in the studies concerning with this problem.

EXPERIMENTAL

I. Change of the vitamin A in fish viscera during storage

(1) *Determination of vitamin A in mackerel viscera*

Sample for this experiment were liver and pyloric caeca of mackerel which had been caught in the offing of Izu-Oshima, 34~38 cm. in length, 490—760g in weight.

The content of vitamin A was measured with the process decribed in the preliminal report[8], but in this method the vitamin A in viscera are not completely extracted, for the reason of the existence of compounds insoluble in chloroform such as vitamin A-protein complex. On the other hand, the alkali digestion method is excellent to the extraction efficiency of vitamin A from viscera in comparison of the former method (Table 1). But in this experiment we determined vitamin A potency by the former method — the direct extraction by chloroform. The details of both methods will be described in the next report.

Table 1. Inffuence of the extraction method on vitamin A in mackerel viscera. a — direct chloroform extraction method; b — alkali digestion method; p. c. oil — pyloric caeca oil.

Sample number	Vitamin A potency in liver oil		Vitamin A potency in p. c. oil	
	a	b	a	b
1	3,380	9,570	390	420
2	500	710	trace	310
3	340	500	130	147

(2) *The content of vitamin A in mackerel viscera and its change during storage*

(a) *Treatment of sample:* Ground liver or pyloric caeca removed immediately after landing at a market, and transfered in the weighing bottle from which 0.5–1.0 g. of sample should be taken out at a certain period of storage, and then determined the content of vitamin A by the process above mentioned.

(b) *Results:* The loss of vitamin A in liver, stored at room temperature (5°~30°C), was less in 100 hours after the catch, but at the passing of 100 hours the loss of vitamin A increased rapidly (Table 2, 3, 4, 5 and Fig. 1).

At 37°C, the vitamin A was so much destroyed as indicating the loss of 70~85 percentages during 35 hours (Table 6 and Fig. 2).

In the pyloric caeca, vitamin A potency dropped more rapidly than the liver, It may be rich in an enzyme to destory the vitamin A. There was

distinctly no relations between the sex of fish and the loss of vitamin A in viscera, except two female samples which gave more losses of vitamin A than the female samples.

From these experimental datas, we find to increase the vitamin A content in liver at a certain stage in storage. This fact may be due to liberate the vitamin A by the enzyme action from the complex with protein insoluble in chloroform.

In view of this fact, it is shown that at lower temperature the vitamin A in viscera less drops its potency for a long time after the catch of fish, but in the raising of temperature as 37°C the loss of vitamin A increases rapidly.

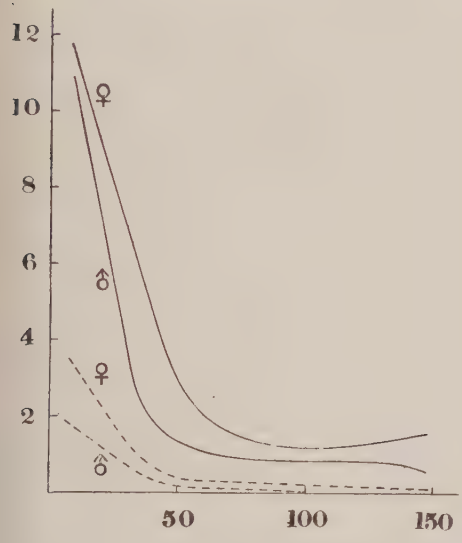


Fig. 1. The loss of vitamin A in mackerel during storage at 10°C. Ordinate, vitamin A content in 1 g. of liver (I.U.×1000); Abscissa, hour of storage.——, Liver; - - - -, Pyloric caeca.

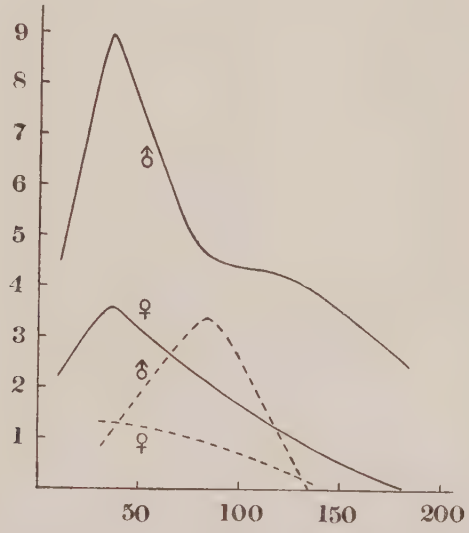


Fig. 2. The loss of vitamin A in mackerel viscera during storage at 37°C. Ordinate, vitamin A content in 1 g. of liver (I.U.× 1000); Abscissa, hour of storage;——, Liver; - - - -, Pyloric caeca.

Table 2. The loss of Vitamin A in mackerel viscera during storage.

Ttorage at 10°C (hrs.)	Liver (I. U.)		Pyloric caeca (I. U.)	
	Female	Male	Female	Male
12	4,660	2,350	—	—
36	8,930	3,570	1,090	1,300
84	4,630	2,100	3,440	930
132	4,050	920	175	190
180	2,500	trace	—	—

Table 3. The loss of vitamin A in mackerel viscera during storage.

Storage at 10°C (hrs.)	Liver (I. U.)		Pyloric caeca (I. U.)	
	Female	Male	Female	Male
60	6,080	1,640	1,970	1,300
108	7,220	2,390	1,470	970
156	1,130	84	441	140

Table 4. The loss of vitamin A in mackerel viscera during storage. A: 36.0cm in body-length, 636g in body-weight, 11.1g in weight of liver, 11.6g in weight of pyloric caeca. B: 34.3cm in body length, 492g in body weight, 7.7g in weight of liver, 10.5g in weight of pyloric caeca.

Storage at 20°C (hrs.)	Liver (male)		Pyloric caeca (male)	
	A	B	A	B
14	1,720	2,940	1,940	760
34	1,260	3,720	1,010	760
70	2,200	2,880	250	340
100	970	1,970	330	270
124	840	3,020	290	—
146	—	1,970	170	—

Table 5. The loss of vitamin A in mackerel viscera during storage. 37.8cm in body length, 757g in body weight, 6.5g in weight of liver, 12.1g in weight of pyloric caeca.

Storage at 20°C (hrs.)	Liver	Polyric caeca	Storage (days)	Liver	Pyloric caeca
12	13,300	2,100	10	10,260	870
24	12,670	1,890	12	6,080	500
44	17,100	2,730	14	7,600	740
94	12,620	2,100	18	5,206	420
121	8,740	756	21	4,940	380
144	11,400	840	24	4,104	380
164	7,980	1,340			

II. On the vitamin A potency in liver oil and its stability depending on the difference of the extraction methods

(1) *Great blue shark liver oil*

Samples were taken from a same lot, and produced every liver oil by two methods, the alkali digestion and autolysis. The vitamin A was determined by the absorption spectra with a Beckmann photometer using isopropyl alcohol as a solvent.

Results: The figures given in Table 7 shows the losses of vitamin A in the liver oils produced by the alkali digestion and the autolysis method. It

will be seen from the figures that the former contains more amounts of vitamin A than the latter. In addition to this fact, it has been evidently observed that the liver oil obtained by the former method was more excellent than the latter in the stability of vitamin A.

Table 6. The loss of vitamin A in mackerel viscera during storage at 37°C. Male: 37.8 cm in body length, 74.5 g in body weight, 16.4 g in liver weight, 13.0 g in pyloric caeca weight. Female: 37.5 cm in body length, 712 g in body weight, 6.1 g in liver weight, 12.2 g in pyloric caeca weight.

Storage	Liver		Pyloric caeca	
(hrs.)	Male	Female	Male	Female
10	10,260	11,400	1,621	3,360
45	1,550	3,740	226	504
69	1,040	1,680	50	240
95	880	1,260	20	250
120	840	1,260	—	200
144	590	1,560	—	50

Table 7. Influence of extraction method on the vitamin A potency in great blue shark liver oil.

Extraction method	V-A (I. U.)	300/328 mμ	V-A (I. U.)	300/328 mμ	loss of V-A
Alkali digestion	13,870	72.2	13,800	72.4	0.51 %
Antolysis	9,920	69.7	9,480	74.0	4.44 %

(2) *Mackerel liver oil*

Procedure: Ground the mackerel livers and digested the liver tissue through the heating after the control of liquid to pH 9~10 with 15 % NaOH solution. Cotton seed oil was added into the digestion liquid corresponding to 10 % weight of liver, and continued the heating of liquid stirring. After 30 minutes, the liver oil was separated from the digestion liquid diluted to 10 times with hot water, and was washed twice with hot water and dehydrated with anhydrated sodium sulfate.

Procedure in autolysis method: Controlled to pH 6.0 the ground liver, digested the liver tissue with the proteolytic enzyme in liver at one hour at 60°C, and then continued the digestion for 4 hours after addition of cotton seed oil as the extractive oil for vitamin A. At the end of extraction, added the abundant hot water to separate the ilver oil.

When the digestion liquid is diluted and liver oil is not separated owing to the producing of strong emulsion, we must separate it by the other extraction method.

Determination of the vitamin A was carried on the absorption spectrum method.

Results: The mackerel liver oil obtained by the alkali digestion method gave the vitamin A value and the extraction ratio (300/328 m μ), as much as the ordinary market liver oils have. On the contrary, the liver oil separated by the autolysis method had very lower potency.

The extinction ratio of vitamin A gave always in each experiment the value over 1.00; in a extreme case it was 5.00, against the average value of 0.60~0.65 for the market liver oil. Therefore, in such a high value of the extinction ratio, the vitamin A potency in liver oil dropped so much as the vitamin A was no more measured, since it might be changed into other compounds than vitamin A (Table 8).

Table 8. Influence of extraction method on vitamin A in mackerel liver oil.

Sample number	Date of analysis	Extraction method	Vitamin A in liver oil		extinction ratio (×100)
			$E\ \frac{1\%}{1\ cm}\ 328$	I. U.	
1	May, 30	alkali d.	19.05	36,100	65.1
2	June, 12	autolysis	2.06	3,890	138.8
3	〃 , 19	alkali d.	29.37	55,600	64.0
4	〃 , 20	autolysis	0.25	460	549.5
5	〃 , 〃	〃	0.30	560	278.0
6	〃 , 21	〃	5.52	10,440	169.4

Principle in the process of extraction: 1—extraction with cotton seed oil; 2—autolysis for 5 days in room temperature; 3—extraction with cotton seed oil; 4—autolysis for 5 hours at 60°C; 5—reextraction wit oil the residue of No. 4; 6—extraction with ether.

The datas above mentioned have been given using the samples which were stored comparatively for long time after the catch. Such experiments are often caused in the error which may be derived from the freshness of sample. For this reason an experiment was again carried out using a fresh sample.

As shown in Table 9, 10 and Fig. 3, the vitamin A in liver and pyloric caeca were almost destroyed in the autolysis at 60°C for 4 hours, and we observed that the liver was mostly oxidized at such a condition in judging the Kries-test.

Table 9. Effect of autolysis on the vitamin A potency of liver oils of mackerel and skipjack.

Wave length	No. 1		No. 2		No. 3		No. 4		No. 5	
(mu)	Optical density	$E^{1\%}_{1cm}$	Optical density	$E^{1\%}_{1cm}$	Optical density	$E^{1\%}_{1cm}$	Optical density	$E^{1\%}_{1cm}$	Optical density	$E^{1\%}_{1cm}$
(A) Whole oil										
300	33.5	0.084	21.5	0.054	357	0.89	244	0.61	568	1.42
310	25.0	0.063	15.0	0.038	419	1.05	300	0.75	728	1.82
320	18.5	0.046	9.0	0.023	441	1.10	330	0.83	820	2.05
325	18.5	0.046	9.0	0.023	458	1.15	344	0.86	857	2.14
328	16.0	0.040	7.0	0.018	451	1.13	340	0.85	849	2.12
334	15.0	0.038	5.0	0.013	399	1.00	304	0.76	759	1.90
350	7.5	0.019			233	0.58	180	0.45	430	1.08
(B) Unsap. matters.										
300	725	2.41	433	1.44	314	3.54	165	1.80	609	9.23
310	370	1.23	200	0.79	334	3.78	174	1.90	761	10.53
320	185	0.62	90	0.30	351	3.97	182	1.98	880	13.33
325	150	0.50	71	0.24	357	4.04	185	2.02	939	14.23
328	136	0.45	64	0.21	354	4.00	180	1.96	922	13.99
334	114	0.38	52	0.17	325	3.68	165	1.80	872	13.21
350	66	0.22	28	0.09	202	2.28	100	1.09	548	8.30
(C) Fatty acid										
300	480				83					
310	490				85					
320	480				83					
325	480				82					
328	470				80					
334	426				72					
350	296				46					

No. 1—oil obtained from the mackerel liver autolysed for 5 hour at 60°C.

2—oil obtained through the alkali digestion of the residue on No. 1 extraction.

3—oil obtained from the skipjack liver autolysed for 2.5 hours at 60°C.

4—oil obtained from the skipjack liver autolysed for 5.0 hour at 60°C.

5—oil obtained by the alkali digestion of skipjack liver.

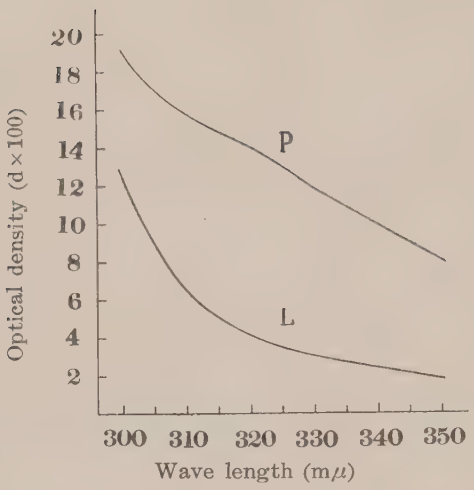


Fig. 3. Vitamin A potency in mackerel viscera oils obtained by autolysis.
L—liver oil
P—pyloric caeca oil

Table 10. Effect of autolysis on the vitamin A potency of liver oil of mackerel.

Wave length (mμ)	Liver		Pyloric caeca	
	Optical density	$E\ \frac{1\ \%}{1\ cm}$	Optical density	$E\ \frac{1\ \%}{1\ cm}$
300	0.124	0.449	0.190	1.10
310	0.065	0.235	0.157	0.909
320	0.0395	0.143	0.140	0.811
325	0.036	0.131	0.1295	0.75
328	0.033	0.1195	0.124	0.718
334	0.0285	0.103	0.110	0.636
350	0.0190	0.068	0.08	0.463
300/328	226.0		153.3	
350/328	57.6		64.5	
U.S.P. Unit at 325 mμ	226		1360	

(3) *Skipjack liver oil*

Results obtained from the mackerel liver oil may be given similarly in the skipjack (*Katsuwonus pelamis*) liver oil. We observed this phenomenon with the process as same as in mackerek.

Results: As shown in Table 9, 10, 11 and Fig. 4, the skipjack liver oil did not posses such specifities as shown in mackerel that the mackerel liver oil was oxidized directly by an enzyme action during autolysis.

On the contrary, the mackerel liver oil which observed pararelly, gave so much losses of vitamin A as its E value could not be determined, and it gave strong Kreis reaction.

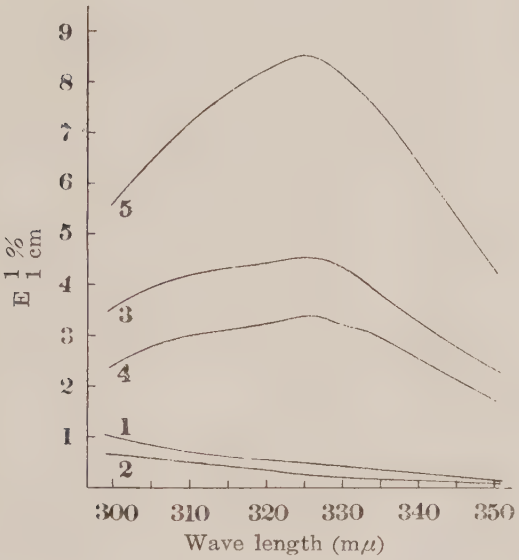


Fig. 4. Vitamin A potency of mackerel liver oil (whole oil).

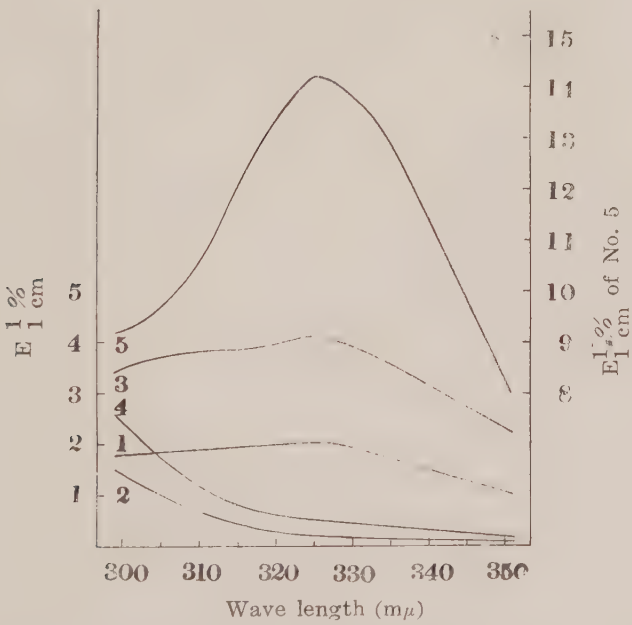


Fig. 5. Vitamin A potency of mackerel and skipjack liver oil (unsap. matt.).

1 Oil obtained from the mackerel liver autolysed for 5 hrs. at 60°C.
2 Oil obtained through the digestion of the residue on No. 1 extraction.
3 Oil obtained from the skipjack liver autolysed for 2.5 hrs. at 60°C.
4 Oil obtained from the skipjack liver autolysed for 5 hrs. at 60°C.
5 Oil obtained by the alkali digestion of skipjack liver.

Addition to this observation, the datas were given by the unsaponifiable substances in skipjack liver oil. As shown Fig, 5 and Table 11, it was resulted as same as in the whole oil. From these two experiments, it would be introduced the fact that the mackerel liver oil gave the maximum absorption sectrum at a point of more shorter wave length than 300 mμ for the autolysis of liver, in spite of the maximum absorption spectrum at 328 mμ of the skipjack liver oil.

(4) *Absorption spectra of the mixed fatty acids in the liver oil obtained from autolysed liver*

The speciality, indicated in the absorption spectra of mackerel liver oil, may be not only due to the oxidation of the vitamin A but of the unsaturated fatty acids in liver oil. From this assumption we determined the absorption spectrum of mixed fatty acids separated from the oxidized mackerel liver oil.

Results: As shown in Table 9 and Fig. 6 the oxidation of the mixed fatty acids in the mackerel liver oil was not carried on, and resulted also similarly in the skipjack liver oil. It is easily understood in comparison with both absorption curves. According to these experiments, it was proved that the speciality in the absorption spectrum of mackerel liver oil obtained from autolysed liver, is not resulted in the oxidation of unsaturated fatty acid but the vitamin A.

II. Stability of liver oil produced by the autolysis method

The liver oils produced by the autolysis method have less stability than the ones produced by the alkali digestion method. However, this stability may be somewhat differed from the sort of liver or the digestion method. At such a view point, we carried on the following experiments.

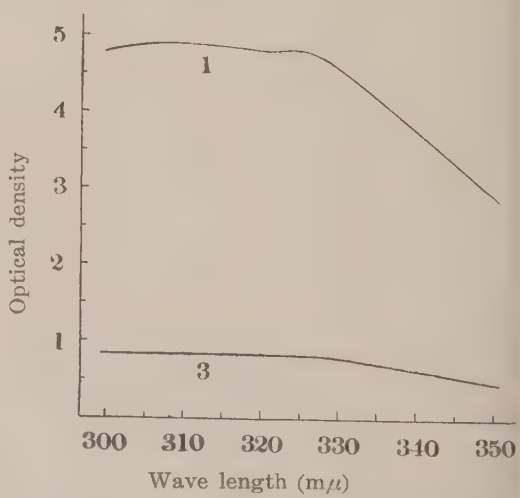


Fig. 6 The optical density of the fatty acids in liver oil

Experiment:

(a) Poured into a bottle small amount of skipjack liver oil which gave little the Kreis reaction, and stirred the bottle aerating for 2 hours at room temperature, and then repeated twice this treatment and used the sample after the exposition in air for ten days.

(b) After the autolysis of the ground skipjack liver at 60°C for 4 hours, extracted the fatty substances in liver with ether. Control was prepared by the alkali digestion method. These two samples were stored at room temperature of 25~30°C after pouring into the bottle with wide stopper in one third volume. At a certain time after storage, the vitamin A potency was determined.

Table 11. Effect of autolysis on the vitamin A potency of liver oil of skipjack.

Wave length (mμ)	Alkali-digestion		Autolysis for 4 hours		Autolysis for 8 hours		Autolysis for 8 hours (oxide)	
	Optical density (×1000)	$E^{1\%}_{1\,cm}$	Optical density (×1000)	$E^{1\%}_{1\,cm}$	Optical density (×1000)	$E^{1\%}_{1\,cm}$	Optical density (×1000)	$E^{1\%}_{1\,cm}$
(A) Whole oil								
300	102	5.70	126.5	4.62	296	3.39	387	3.32
310	109	6.09	140.5	5.13	324	3.79	256	2.21
320	111	6.65	152.5	5.50	355	4.15	170	1.46
325	122	6.82	157.0	5.73	364	4.25	145	1.24
328	122	6.82	157.0	5.73	364	4.25	135	1.12
334	115	6.43	145.0	5.30	335	3.92	109	0.94
350	81	4.50	93.5	3.41	215	2.51	65	0.55
300/328	83.7		80.6		79.7		296.5	
(B) Unsap. matter								
300	500	4.32	238	3.46	235	2.68	226	0.93
310	622	5.38	293	4.26	293	3.34	185	0.76
320	706	6.10	324	4.71	325	3.71	157	0.65
325	740	6.38	338	4.92	341	3.99	145	0.60
328	734	6.34	333	4.84	334	3.81	139	0.57
334	677	6.11	300	4.36	300	3.42	124	0.51
350	447	3.86	178	2.59	175	2.00	89.5	0.37
f	792		842		895		—	
K-test					negative		strong	

Results:

On the experiment (a): As shown in Table 11 and Fig. 7, the sample gave as the similar results in absorption spectrum as the mackerel liver oil extracted from the autolysed liver. From this result we had our opinion that the extinction ratio (300/328mμ) of all liver oils increase the value, in accordance with the oxidation of liver oil.

On the experiment (b): The stability of skipjack liver oil prepared by digestion for 4 hours was less than the other preparations, and the induction period was only for twenty days. On the contrary, the preparation in the autolysis for 8 hours had so high stability as the liver oil obtained from the

materials digested with alkali (Table 12 and Fig. 8). This fact will be proved in coming observations.

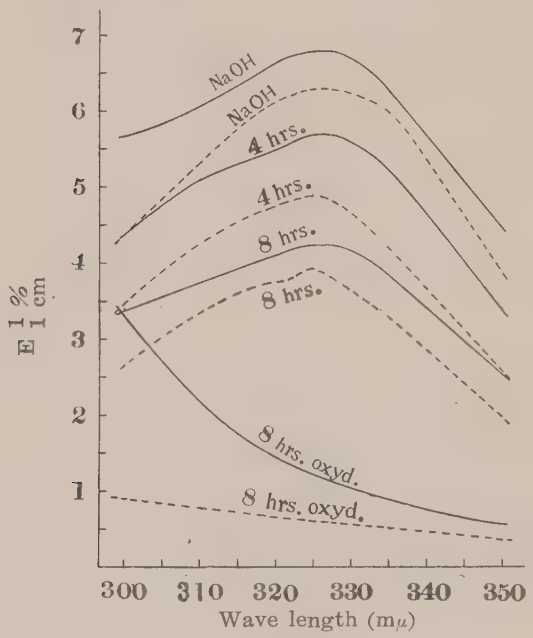


Fig. 7

Fig. 7. Influence of digestion methods on the vitamin A potency of skipjack liver oil. whole oil; - - - - - unsap. matt.

Fig. 8. The optical change of skipjack liver oil during storage. a, the value measured immediately after preparation. b, the value measured after 16 days storage. c, the value measured after 28 days storage. d, the value measured after 30 days storage.

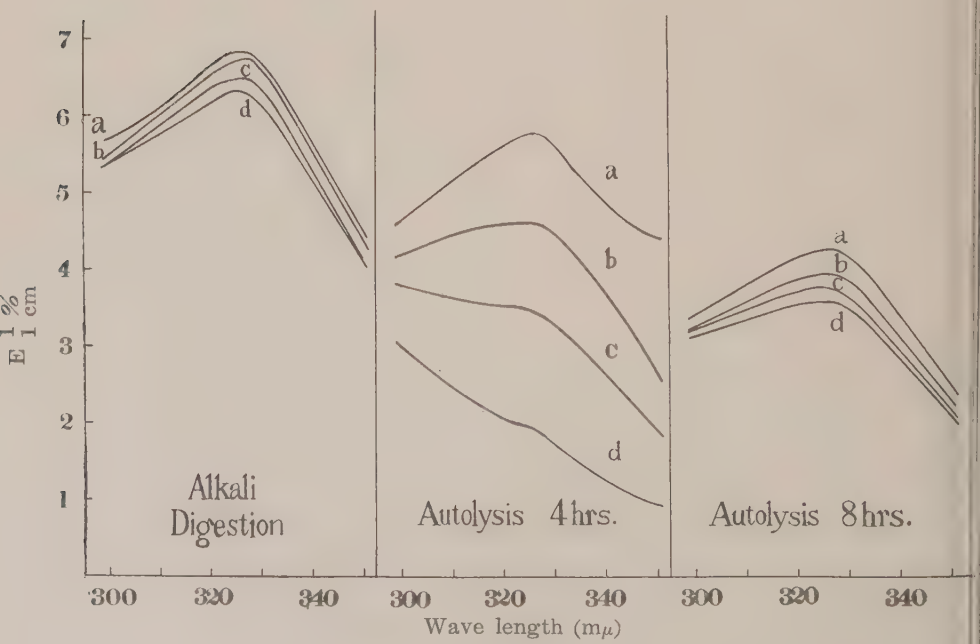


Fig. 8

Table 12. Stability of skipjack liver oil.

Wave length (mμ)	Alkali digestion, $E\frac{1\%}{1\text{ cm}}$				Autolysis (4 hrs.), $E\frac{1\%}{1\text{ cm}}$				Autolysis (8 hrs.), $E\frac{1\%}{1\text{ cm}}$			
	0 day	16 days	23 days	30 days	0 day	16 days	23 days	30 days	0 day	16 days	23 days	30 days
300	5.70	5.50	5.38	5.38	4.62	4.17	3.76	2.99	3.39	3.25	3.21	3.11
310	6.09	5.98	5.86	5.74	5.13	4.43	3.62	2.48	3.79	3.57	3.47	3.34
320	6.65	6.52	6.36	6.16	5.56	4.56	3.51	2.06	4.15	3.86	3.71	3.54
325	6.82	6.68	6.48	6.32	5.73	4.59	3.46	1.93	4.25	3.94	3.77	3.58
328	6.82	6.76	6.48	6.24	5.73	4.56	3.38	1.85	4.25	3.91	3.72	3.56
334	6.43	6.34	6.08	5.88	5.30	4.18	3.04	1.59	3.92	3.60	3.39	3.27
350	4.50	4.40	4.14	4.01	3.41	2.66	1.91	0.94	2.51	2.30	2.18	2.10
300/328	83.7	81.5	83.2	84.6	80.6	91.6	111.0	162.3	79.7	83.2	86.1	87.2
350/328	66.0	65.2	94.0	64.2	59.6	58.4	56.5	50.9	59.1	58.8	58.6	59.0

III. The absorption spectra of oxidized liver oils

The mackerel liver oil prepared by the autolysis method or the other liver oils oxidized artificially, vary the optical density in ultraviolet light inaccordance with the promotion of oxidation, and the maximum absorption spect, rum will be flattered at apoint of shorter wave length than 300 mμ. Howeverwe could not determined what type of absorption curve had the liver oil.

In concerning with the vitamin A epoxide — the oxidation product of vitamin A, Karrer[9], Mails[10] and Swern[11] have reported on the occurence and the absorption spectrum, and then Hawkins and Hunter[12] and Embree[13] have showed the oxidation product of vitamin A in the name of subvitamin A.

From those studies, it is assumed that the oxidation product of vitamin A may have the maximum absorption in the regions below 300 mμ, and Swern observed the absorption of vitamin A epoxide with a marked flattening of the curve at 272 mμ.

The oxidized mackerel liver oil have not yet illustrated the proper absorption curve of vitamin A epoxide in our experiments.

SUMMARY

(1) The loss of vitamin A in mackerel liver oil was less during storage at lower temperature below 30°C, but increased rapidly at a temperature above 37°C.

(2) The mackerel liver oil extracted with ether from the digestion substances of liver which autolized for 2.5~5.0 hours at 60°C after the addition of some cotton seed oil as solvent, diminished markedly the vitamin A potency. On the contrary, the mackerel liver oil obtained with the alkali digestion method had no loss of vitamin A during extraction compared with the autolysis method.

The skipjack liver oil treated with same process as mackerel had no diminition of vitamin A potency.

(3) The mackerel liver oil prepared with the autolysis method or the skipjack liver oil oxidized with aeration, gave together a strong Kries reaction and the vitamin A potency, the value of $E\ {}^{1\%}_{1\,cm}$, diminished so markedly that it was not hardly determined. The absorption spectra of these oxidized liver oils may be flattered the peaks of curves in the region below 300 mμ.

(4) The absorption curves of mixed fatty acids seprated from the liver oils of mackerel and skipjack were distinctly different from the curves obtained with the whole oils, and the mixed fatty acids gave no effect on the absorption spectra of vitamin A or vitamin A epoxide. For this reason, it was assumed the increase in the extinction coefficiency in absorption spectrum (300/328 mμ) — the loss of vitamin A — is independent of the unsaturated fatty acid in oxidized liver oil.

(5) There were no oxidation based on oxygen or other active inorganic substances. In the autolysis of mackerel liver but on the catalytic action of an enzyme as lipoxidase or the other analogues. This suggestion would be demonstrated in the coming experiments

Concluding this report, we wish to express our thanks to Mr. Suda, a member of the measurement institute of Welber Eris Co. Ltd., for measurement of absorption spectrum, and to the financial aid given by the Ministry of Education.

LITERATURE

(1) J. B. Sumner and R. T. Sumner; J. Biol. Chem., **134**, 531 (1940).
(2) H. H. Strain; J. Am. Chem. Soc., **63**, 3542 (1941).
(3) A. K. Balls, B. Alelrod and M. W. Kils; J. Biol. Chem., **149**, 491 (1941).
(4) C. H. Lea; J. Soc. Chem. Ind., **56**, 376 (1937).
(5) A. J. Banks; J. Soc. Chem. Ind., **56**, 13 (1937).
(6) E. Hove; Science, **98**, 433 (1943).
(7) H. Theorell, R. Holman and A. Akeson; Arch. Biochem., **14**, 250 (1947).
(8) T. Ono and T. Sato; J. Tokyo Univ. Fish., **38**, (No. 3), 308 (1952).
(9) P. Karrer and Tucker; Helv. Chem. Acta., **28**, 717 (1945).
P. Karrer; ibid. **28**, 474 (1945).
(10) N. H. Milas; Vitamins and Hormones, **5**, 138 (1947).
(11) D. Swern; J. Am. Chem. Soc., **69**, 1692 (1947).
(12) E. Hawkins and R. Hunter; Biochem. J., **38**, 34 (1944).
(13) N. D. Embree and E. M. Shantz; J. Am. Chem. Soc.. **65**, 906 (1943).

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

DEHYDRATION OF THE AGAR-AGAR GEL
Part 3. DEHYDRATION BY PRESSURE

Ikunosuke Okada, Isamu Osakabe, Yuji Kuratomi, Takeaki Kikuchi.

(Received on July 1, 1952)

None has reported on the dehydration of the agar-agar gel by the pressure with the exception of the patent registered by Imai[1] and Ide[2]. The precise results on the pressure method has not been reported up to date. Then the authors tried to ascertain the dehydration of the agar-agar gel by the pressure in detail. The method brought about a very good result and will be available for the industrial purpose.

EXPERIMENTAL

Several kinds of the agar-agar gel were prepared from the following seaweeds, *Gelidium Amansii* Lams., *Gracilaria Confervoides* (L.) Grev., and the alkali treated *Gracilaria Confervoides* (L.) Grev. under the usual method, then the gel was cut into thin slices. The following experiments were tried by the use of this agar-agar gel.

1. Dehydration of the agar-agar gel by the difference of the pressure.
2. Dehydration of the agar-agar gel due to the difference of its concentrations.
3. Dehydration of the agar-agar gel by the influence of its thickness.
4. Comparison of the agar-agar gel by the difference of its species of the seaweeds.
5. Comparison of the dehydration of this pressure method and the adsorption method by "Ton platte".
6. The yield of the agar-agar prepared by this method was compared with the other method e. g. refrigiration, "Ton platte", and cataphoresis.
7. Ingredients of the agar-agar prepared by this pressure method.
8. Emperical formula of the residual water in the agar-agar gel.

The agar-agar gel containing 1% and 2% of the agar-agar gels were prepared from the above mentioned seaweeds. In this experiment, the agar-agar gel was sliced into 10cm^2. and 0.5 cm. in thickness, and 10 cm^2. and 1.0 cm. in thickness. Then the sliced gel was tightly wrapped in a thin and strong cloth.

1. Dehydration of the Agar-Agar Gel by the Difference of the Perssure

The agar-agar gel wrapped in a cloth was pressed under the various pressures e. g. 1 kg/cm^2., 0.3 kg/cm^2., 0.2 kg/cm^2., and 0.1 kg/cm^2. The agar-agar gel dehydrated by the pressure was weighed each hour, and the percentage of the dehydration was calculated. The results were shown on the table 1–6 and the Fig. 1–11. As the above results show, it can be readily observed that the higher the pressure was, the more the dehydration was achieved. As shown on the table 1, and the Fig. 1, the gel containing the 1% agar-agar extracted from *Gelidium Amansii* Lams. was dehydrated under the pressure of 1 kg/cm^2, 84.1% after 30 minutes, and 92.0% after 5 hours, but under the pressure of of 0.1 kg/cm^2. it was dehydrated only 45.9% after 30 minutes and 77.3% after 5 hours.

Table 1. Dehydration of the agar-agar gel (0.5 cm. and 1.0 cm. in thickness) containing 1% agar-agar prepared form *Gelidium Amansii* Lams.

Thickness of the gel	Pressure / Hour	1.0 Kg/cm^2	0.3 Kg/cm^2	0.2 Kg/cm^2	0.1 Kg/cm^2
cm		%	%	%	%
0.5	0.5	84.1	73.7	60.0	45.9
	1.0	87.2	82.2	76.8	62.1
	1.5	89.4	85.3	81.1	72.2
	2.0	91.0	86.4	83.5	75.0
	3.0	91.6	87.5	84.8	76.4
	5.0	92.0	88.6	85.8	77.3
1.0	0.5	80.5	65.1	44.4	21.1
	1.0	88.0	78.2	59.6	33.0
	1.5	89.1	82.4	67.9	40.9
	2.0	89.3	84.0	72.0	46.1
	3.0	90.3	85.1	74.2	49.2
	5.0	90.5	86.8	75.1	51.5

Table 2. Dehydration of the agar-agar gel (0.5 cm. and 1.0 cm in thickness) containing 2% agar-agar prepared from *Gelidium Amansii* LAMS.

Thickness of the gel	Pressure / Hour	1.0 Kg/cm²	0.3 Kg/cm²	0.2 Kg/cm²	0.1 Kg/cm²
cm		%	%	%	%
0.5	0.5	78.2	44.5	34.0	20.1
	1.0	84.2	59.1	48.5	29.6
	1.5	86.9	68.2	59.2	36.3
	2.0	89.0	71.3	63.6	41.0
	3.0	90.8	75.4	69.0	45.6
	5.0	91.5	77.0	75.1	48.3
1.0	0.5	63.2	26.3	19.2	10.9
	1.0	76.8	37.1	28.7	17.8
	1.5	79.0	46.1	36.0	21.8
	2.0	81.1	51.6	41.9	25.0
	3.0	84.3	60.0	46.8	27.2
	5.0	87.0	68.8	50.4	30.4

Table 3. Dehydration of the agar-agar gel (0.5 cm. and 1.0 cm in thickness) containing 1% agar-agar prepared from the alkali treated *Gracilaria Confervoides* (L.) GREV.

Thickness of the gel	Pressure / Hour	1.0 Kg/cm²	0.3 Kg/cm²	0.2 Kg/cm²	0.1 Kg/cm²
cm		%	%	%	%
0.5	0.5	93.5	87.8	83.5	77.3
	1.0	94.6	90.0	89.5	85.0
	1.5	95.2	91.7	91.3	88.1
	2.0	95.9	94.3	92.3	90.0
	3.0	96.2	94.7	92.8	91.6
	5.0	96.8	94.7	93.1	92.3
1.0	0.5	91.4	86.9	72.2	52.2
	1.0	93.4	88.0	86.5	67.4
	1.5	93.8	90.3	90.1	77.5
	2.0	94.1	92.9	90.8	83.5
	3.0	94.5	93.2	91.4	84.2
	5.0	94.8	93.5	91.9	84.6

Table 4. Dehydration of the agar-agar gel (0.5 cm. and 1.0 cm. in thickness) containing 2% agar-agar prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev.

Thickness of the gel	Pressure / Hour	1.0 Kg/cm²	0.3 Kg/cm²	0.2 Kg/cm²	0.1 Kg/cm²
cm		%	%	%	%
0.5	0.5	87.5	79.4	72.3	50.0
	1.0	90.5	85.5	81.9	68.2
	1.5	92.5	88.3	84.8	73.4
	2.0	92.6	88.7	86.2	78.6
	3.0	92.6	88.9	86.9	81.3
	5.0	92.8	90.0	87.5	82.9
1.0	0.5	84.4	71.9	46.8	27.5
	1.0	88.3	80.1	58.2	43.8
	1.5	89.6	85.0	67.9	51.6
	2.0	90.6	85.1	76.8	58.7
	3.0	91.5	87.5	79.4	62.0
	5.0	92.0	88.4	81.3	65.2

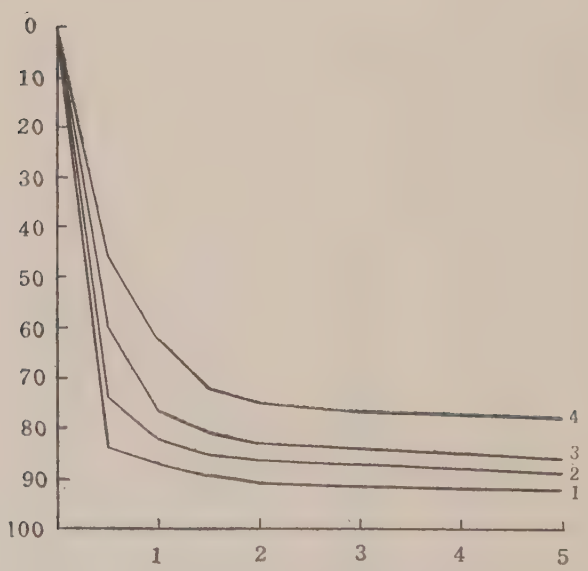


Fig. 1. Dehydration of the gel containing 1% agar-agar prepared from *Gelidium amansii* Lams. (0.5 cm in thickness).

Ordinate—dehydration ratio; Abscissa—hour.

1.—1 kg/cm² pressure; 2—0.3 kg/cm² pressure;

3.—0.2 kg/cm² pressure; 4—0.1 kg/cm² pressure.

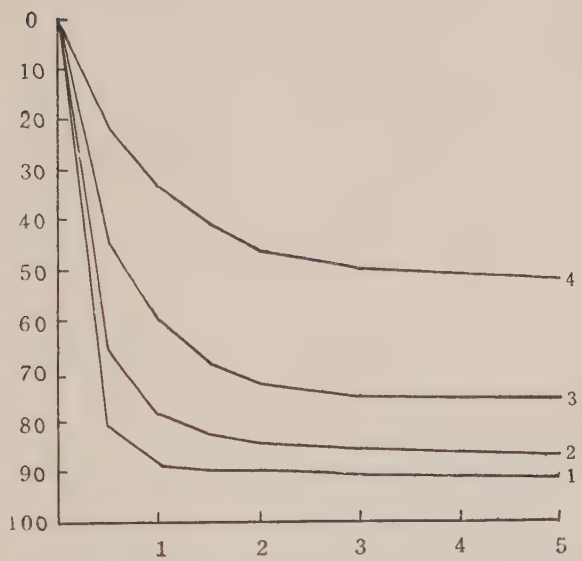


Fig. 2. Dehydration of the gel containing 1% agar-agar prepared from *Gelidium Amansii* LAMS. (1.0 cm in thickness).
Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

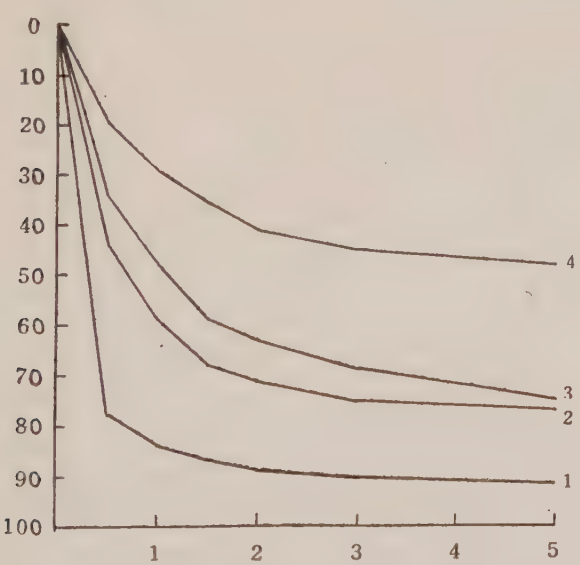


Fig. 3. Dehydretion of the gel containing 2% agar-agar prepared from *Gelidium Amansii* LAMS. (0.5 cm in thickness).
Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

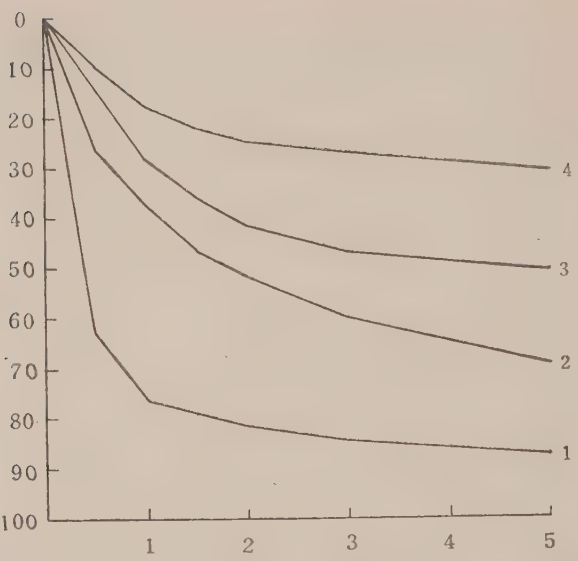


Fig. 4. Dehydration of the gel containing 2% agar-agar prepared from *Gelidium Amansii* Lams. (1.0 cm in thickness).

Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

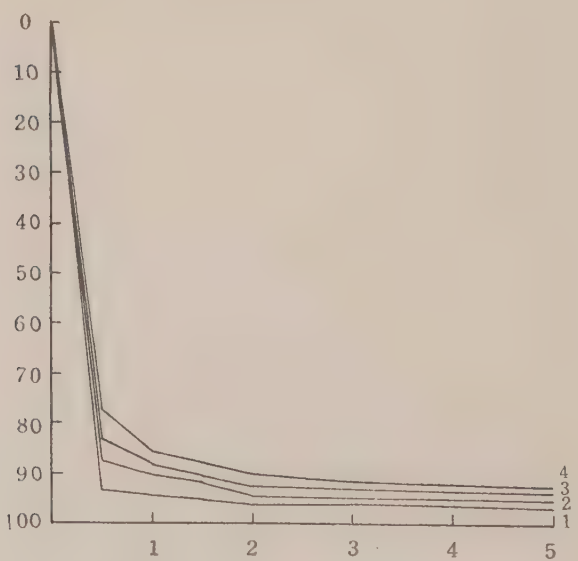


Fig. 5. Dehydration of the gel containing 1% agar-agar prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev. (0.5 cm. in thickness).

Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

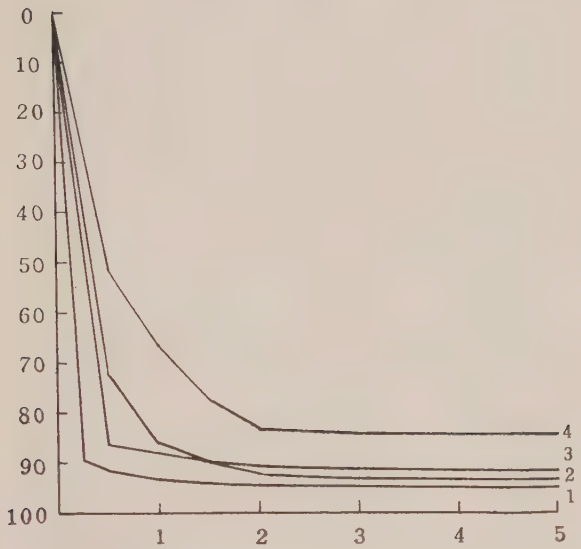


Fig. 6. Dehydration of the gel containing 1% agar-agar prepared form the alkali treated *Gracilaria Confervoides* (L.) GREV. (1.0 cm. in thickness).

Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

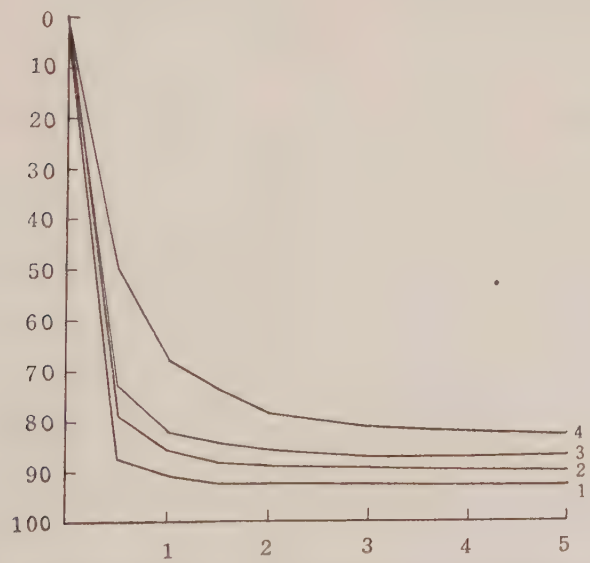


Fig. 7. Dehydration of the gel containing 2% agar-agar prepared from the alkali treated *Gracilaria Gonfervoides* (L.) GREV. (0.5 cm in thickness).

Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

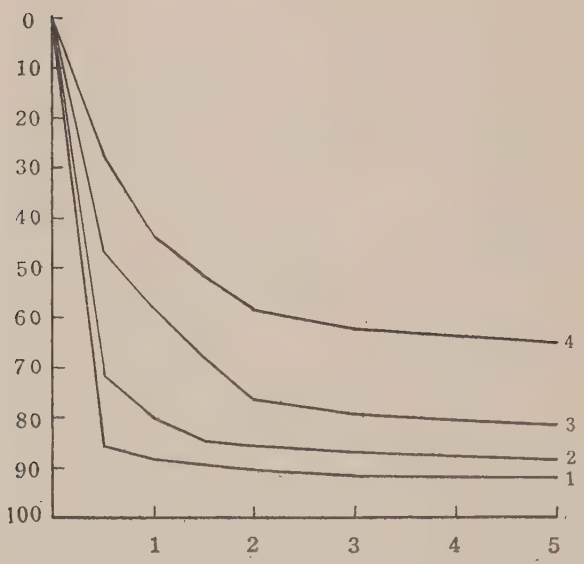


Fig. 8. Dehydration of the gel containing 2% agar-agar prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev. (1.0 cm in tcickness).

Ordinate—dehydration ratio; Abscissa—hour.
1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure;
3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

2. Dehydration of the Agar-Agar Gel due to the Diefference of its Concentrations

According to the tables 1–4 and the Figs. 1–8, the dehydration of the agar-agar gel was considerably influenced by the difference of its concentrations, especially under the lower pressure than the higher. Namely, as seen on the table 1, 2 and the Figs. 1, 3 uuder the 1.0 kg/cm² pressur, the gel containing 1% agar-agar was dehydrated 84.1%, and the gel containing 2% agar-agar was 78.2% after 30 minutes, but under the 0.1 kg/cm² pressure, the gel containing 1% agar-agar was dehydrated 45.9% and the gel containing 2% agar-agar was 20.1% after 30 minutes.

Similar results were obtained as shown on the table 3, 4 and the Fig. 2–8.

3. Dehydration of the Agar-Agar Gel by the Influence of its Thickness

According to the results shown on the table 1–4 and the Fig. 1–8, the dehydration of the agar-agar gel was considerably influenced by the difference

of its thickness, namely the thinner the agar-agar gel was, the more the dehydration was achieved. Especially the gel containing 2% agar-agar was more influenced than that of 1% agar-agar.

As shown on the table 1 and the Fig. 1, 2 under the 1.0 kg/cm² pressure, the gel containing 1% agar-agar and of 0,5 cm in thickness was dehydrated 84.1%, and that of 1.0 cm in thickness was 80.5% after 30 minutes. But under the 0.1 kg/cm² pressure, the gel of 0.5 cm in thickness was dehydrated 45.9%, and that of 1.0 cm in thickness only 21.1% after 30 minutes. Then under the 1 kg/cm² pressure, the gel containing 2% agar-agar and of 0.5 cm in thickness was dehydrated 78.2%, and that of 1.0 cm in thickness only 63.2% after 30 minutes as shown on the table 1 and Fig. 3, 4.

Similar results were obtained as seen on the table 2–5 and Fig. 3–8.

Table 5. Dehydration of the agar-agar gel (0.5 cm and 1.0 cm in thickness) containing 1% agar-agar prepared from the mixed materials of the three species of the seaweeds. (*Gelidium Amansii* LAMS. 30%, *Gracilaria Confervoides* (L.) GREV. 10% the alkali treated *Gracilaria Confervoides* (L.) GREV. 60% by weight).

Thickness of the gel	Pressure / Hour	1.0 Kg/cm²	0.3 Kg/cm²	0.2 Kg/cm²	0.1 Kg/cm²
cm		%	%	%	%
0.5	0.5	89.6	86.4	78.0	68.6
	1.0	96.8	89.5	84.0	79.1
	1.5	92.0	91.0	86.1	81.5
	2.0	92.5	91.6	87.8	82.9
	3.0	93.1	92.2	88.9	86.5
	5.0	94.2	93.1	90.3	89.6
1.0	0.5	87.4	84.0	70.1	50.4
	1.0	89.0	87.1	79.3	61.2
	1.5	90.6	88.9	80.3	69.2
	2.0	90.9	89.9	81.1	74.3
	3.0	91.3	90.4	82.0	76.0
	5.0	92.4	91.0	83.5	78.3

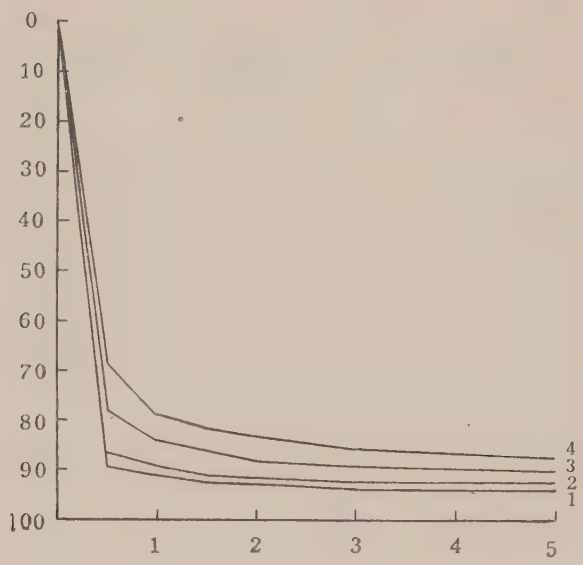


Fig. 9. Dehydration of the gel containing 1% agar-agar prepared from the mixed materials of the follwing three species of the seaweeds. *Gelidium Amansii* Lams. 30%, *Gracilaria Confervoides* (L.) 10%; and alkali treated *Gracilaria Confervoides* (L.) Grev. 60%. (0.5 cm in thickness) Ordinate—dehydration ratio; Abscissa—hour. 1.—1 kg/cm² pressure; 2.—0.3kg/cm² pressure; 3.—0.2 kg/cm² pressure; 4. —0.1 kg/cm² pressure.

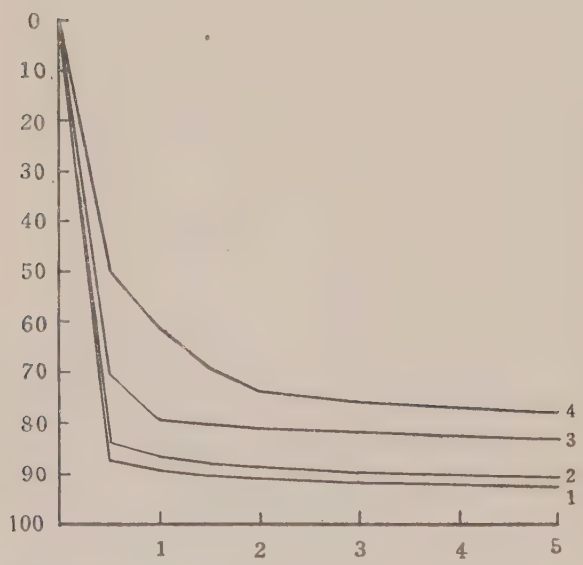


Fig. 10. Dehydration of the gel containing 1% agar-agar prepared from the mixed materials of the three species of the seaweeds. *Gelidium Amansii* Lams. 30%, *Gracilaria Confervoides* (L.) Grev. 10%, and the alkali treated *Gracilaria Confervoide* (L.) Grev. 60%. (1.0 cm in thickness) Ordinate—dehydration ratio; Abscissa—hour. 1.—1 kg/cm² pressure; 2.—0.3 kg/cm² pressure; 3.—0.2 kg/cm² pressure; 4.—0.1 kg/cm² pressure.

4. Comparison of the Dehydration of the Agar-Agar Gel by the Difference of its species of the Seaweeds

Each agar-agar prepared from the different species of the seaweeds posesses its own character, namely, it was observed that the choice of the original materials of the seaweeds have a great influence on the dehydration of the

agar-agar gel by the pressure method. Then in this experiment, the following three samples were used e. g. *Gelidium Amansii* Lams., *Gracilaria Confervoides* (L.) Grev., alkali treated *Gracilaria Confervoides* (L.) Grev., and mixed materials of these three.

There were apparent difference among the dehydrations of the agar-agar gels made from the three original materials above mentioned. According to the table 2 and 4, under the same conditions (1% conc., 0.5 cm in thickness, and 1 kg/cm² pressure) the agar-agar gel prepared from *Gelidium Amansii* Lams. was dehydrated 84.1% after 30 minutes and 92.0% after 5 hours, but the gel prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev. was dehydrated 93.5% after 30 minutes and 96.3% after 5 hours. Then when the alkali treated *Gracilaria Confervoide* (L.) Grev. was mixed as a raw material, the dehydration of the agar-agar gel was greatly facilitated as shown on the table 5 and Fig. 9, 10.

As the above results show, it is a very interesting matter that the agar-agar gel prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev. was very easily dehydrated. Then it may safely recommended with regard to the dehydration of the agar-agar gel by pressure properly to add the alkali treated *Gracilaria Confervoides* (L.) Grev. to the other.

5. Comparison of the Dehydration of the Agar-Agar Gel by this Pressure Method and the Adsorption Method by "Ton Platte"

As the dehydration of the agar-agar gel by pressure was satisfactorily achieved as mentioned above it was compared with the adsorption method by "Ton platte" reported on the part 2[3].

Table 6. Comparison of the dehydration of the agar-agar gel (1% conc., 0.5 cm in thickness) by this pressure method (1.0 kg/cm² pressure) and the "Ton plate" method.

Original materials of the gel	*Gelidium Amansii* Lams.		Alkali treated *Gracilaria Confervoides* (L.) Grev.	
Procedure / Hour	"Ton platte"	Pressure	"Ton platte"	Pressure
	%	%	%	%
0.5	93.0	84.1	96.5	93.5
1.0	94.8	87.2	97.1	94.6
1.5	95.0	89.4	97.5	95.2
2.0	95.5	91.0	98.0	95.8
3.0	96.0	91.6	88.0	96.2
5.0	96.2	92.0	98.0	96.5

As shown on the table 6 and Fig. 11, under the same conditions (1% conc., 0.5 cm in thickness) the "Ton platte" method was a little superior to the pressure method (under 1.0 kg/cm^2 pressure) with respect to the dehydration of the agar-agar gel prepared from *Gelidium Amansii* Lams. But there was no differnce between the two methods with respect to the dehydration of the agar-agar gel prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev.

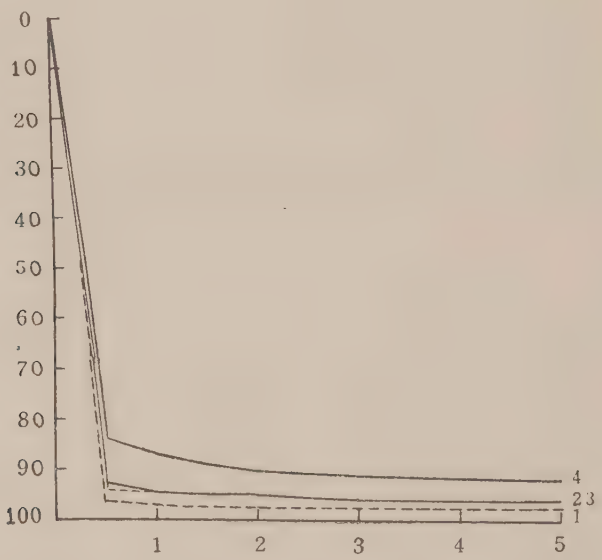


Fig. 11. Comparison of the dehydration of the gel by this pressure method and the adsorption method by "Ton platte". (1% conc. and 0.5 cm in thickness).
Ordinate—dehydration ratio; Abscissa—hour.
1.—"ton platte" method; 2.—pressure method;
3.—"ton platte" method; 4.—pressure method.
Solid line—*Gelidium Amansii* Lams.
broken line— alkali treated *Gracilaria Confervoides* (L.) Grev.

6. The Yield of the Agar-Agar Preared by this Method was Compared with the Other Methods e. g. Refrigiration, "Ton Platte" and Cataphoresis

The yield of the agar-agar prepared from *Gelidium Amansii* Lams. was the highest by the "Ton platte" method and the lowest by the "cataphoresis", as shown on the table 7. but there was no differnce among the yields of the agar-agar prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev.

Table 7. Comparison of the agar-agar yield owing to the difference of the procedures.

Original material of the gel / Procedure	*Gelidium Amansii* LAMS	Alkali treated *Gracilaria Confervoides* (L.) GREV.	*Geldium Amansii* LAMS. 50% Gracilaria Confervoides (L.) GREV. 50%
	%	%	%
"Ton platte"	100.0	100.0	100.0
Refrigiration	97.0	100.0	95.0
Pressure	96.2	100.0	92.0
Cataphoresis	94.5	98.0	90.0

7. The Ingredients of the Agar-Agar Prepared by this Method

The ingredients of the agar-agar prepared by this method were compared with those of the agar-agar dried naturally in the air and made by the other methods, and the results were shown on the table 8.

Table 8. Ingredients of the agar-agar prepared by the different methods.

Ingredients / Procedure of dehydration	Moisture	Ash	Total nitrogen Compound	Jelly strength
Agar-agar	%	%	%	g/cm²
dried in the air	32.0	4.56	1.60	310
pressure	21.3	2.01	0.21	460
"Ton platte"	21.6	1.95	0.15	500
Cataphoresis	19.9	1.35	....	390
refrigiration	22.0	3.10	0.35	420

As shown on the table 8, the contents of the ash and the total nitrogen compound of the agar-agar prepared by this method showed a decrease as compared with the agar-agar dried naturally in the air. The impurities in the agar-agar gel were removed with the water dehydrated, therefore the agar-agar was purified simultaneously with the dehydration. And also with regard to the jelly strength, the pressure method showed the better result.

Although the quality of the agar-agar prepared by this method was excellent, this is a little inferior to the "Ton platte" and cataphoresis method in quality. From the above results, it may be assumed that the pressure method is the most economical from the industrial point of view.

8. Empirical Formula of the Residual Water in the Agar-Agar Gel

When the agar-agar contained y_0 parts in 100 parts of the agar-agar gel, the water was contained $100-y_0$.

If y parts of the water was removed with X hour, for example, on the gel containing 1.0% agar-agar prepared from "*Gelidium Amansii* Lams", it remained 85.1% of water after 30 minuts, 79.2% after 1 hour and so forth, because it exist 99.0% of water at the begining at the dehydration. ($X=0$)

When it was plotted on the logarithmic paper, it showed almost straight line as shown on the Fig. 12 (showed some representative examples), namely, when the residual water was assumed as Z% the relation was represented as follows:—

$$\log_{10}Z=\text{a } \log_{10}X+\text{b or } Z=BX^{A}$$

The roughly estimated value of a and b was shown on the table 9.

Table 9. Roughly estimated value of a. and b.

Kinds of Gel.	Conc. of the Gel. %	Thichness of Gel. cm	Pressure. Kg/cm²	a.	b.
Geridium Amansii Lams.	1.0	0.5	1.0	−0.395	1.045
	1.0	0.5	0.1	* −	* −
	1.0	1.0	1.0	−0.318	1.114
	1.0	1.0	0.1	−0.218	1.806
	2.0	0.5	1.0	−0.489	1.127
	2.0	0.5	0.1	−0.204	1.820
	2.0	1.0	1.0	−0.480	1.362
	2.0	1.0	0.1	−0.121	1.903
Alkali treated *Gracilaria Confervoides* (L.) Grev.	1.0	0.5	1.0	−0.398	0.623
	1.0	0.5	0.1	−0.549	1.146
	1.0	1.0	1.0	−0.230	0.763
	1.0	1.0	0.1	* −	* −
	2.0	0.5	1.0	* −	* −
	2.0	0.5	0.1	−0.494	1.477
	2.0	1.0	1.0	−0.348	0.991
	2.0	1.0	0.1	−0.367	1.740
Mixed materials of the three species of the seaweeds.					
(*Gelidium Amansii* Lams. 30% *Gracilaria confervoides* (L.) Grev. 10%, Alkali treated *Gracilaria Confervaides* (L.) Grev. 60%, by weight).	1.0	0.5	1.0	−0.297	0.9031
	1.0	0.5	0.1	−0.490	1.322
	1.0	1.0	1.0	−0.255	0.982
	1.0	1.0	0.1	−0.403	1.556

* It was not calculated, as the results were not convenient to calcurate.

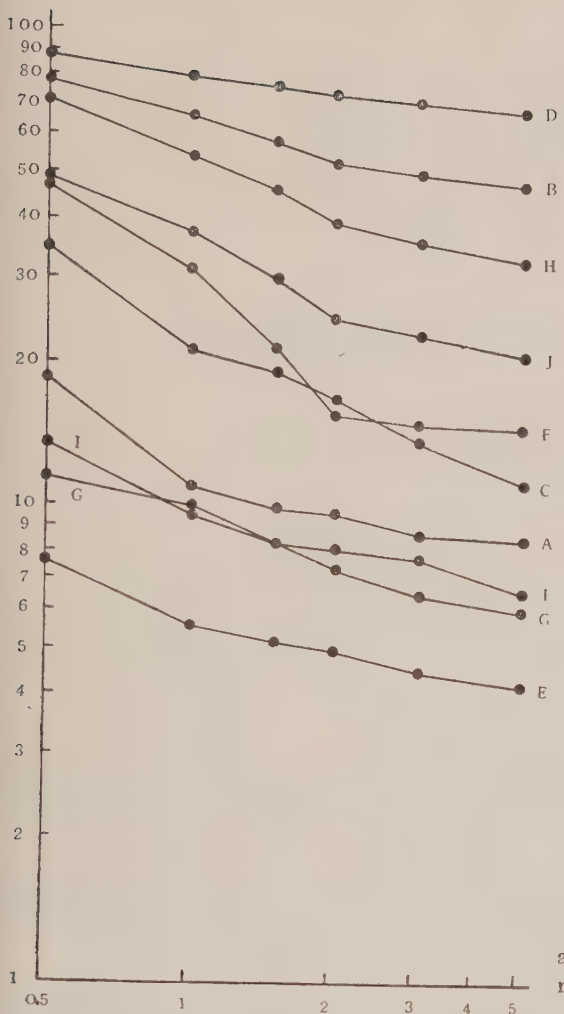


Fig. 12. Residual water in the agar-agar gel. Ordinate—dehydration ratio; Abscissa—hour.

Species of Seaweeds	Concentration	Thickness	Pressure
A.....*Gelidium Amansii* LAMS	1 %	1.0 cm	1.0 kg/cm²
B..... "	1 %	1.0 cm	0.1 kg/cm²
C..... "	2 %	1.0 cm	1.0 kg/cm²
D..... "	2 %	1.0 cm	0.1 kg/cm²
E.....Alkali treated *Gracilaria Confervoides* (L.) GREV.	1 %	1.0 cm	1.0 kg/cm²
F..... "	1 %	1.0 cm	0.1 kg/cm²
G..... "	1 %	1.0 cm	1.0 kg/cm²
H..... "	1 %	1.0 cm	0.1 kg/cm²
I.....Mixed materials of the three species of the seaweeds (*Gelidium Amansii* LAMS. 30%, *Gracilaria Confervoides* (L.) GREV. 10%, Alkali treated *Gracilaria Confervoides* (L.) GREV. 60%, by weight)	1 %	1.0 cm	1.0 kg/cm²
J..... "	1 %	1.0 cm	0.1 kg/cm²

Résumé

1. The dehydration by pressure method was studied on the agar-agar gel prepared from *Gelidium Amansii* Lams., *Gracilaria Confervoides* (L.) Grev., and the alkali treated *Gracilaria Confervoides* (L.) Grev.

2. This pressure method was so excellent that the dried agar-agar was obtained in a short time.

3. The dehydration of the agar-agar gel by this pressure method was considerably influenced by various factors, with reference to the concentrations of the agar-agar, thickness of the gel, and original materials of the gel owing to the species of the seaweeds.

4. The higher the pressure was, the more the dehydration was achieved.

5. The lower the concentration was, the more the dehydration was achieved.

6. The thinner the gel was, the more the dehydration was achieved.

7. The agar-agar gel prepared from the alkali treated *Gracilaria Confervoides* (L.) Grev. was the most easily dehydrated by this pressure method.

8. When the alkali treated *Gracilaria Confervoides* (L.) Grev. was properly combined with the other species of the seaweeds, the dehydration of the gel was considerably facilitated by this pressure method.

9. Although the quality of the agar-agar prepared by this method was excellent, but this method is a little inferior to the "Ton platte" and cataphoresis method in quality.

10. This pressure method was thought to be comparatively economical compared with the other scientific methods with regard to the dehydration of the agar-agar gel, so it will be used for the industrial purpose.

11. Because the dehydration of the agar-agar gel was satisfactorily achieved under the 1.0 kg/cm^2 pressure, it is assumed that any expensive equippment, is not necessary to this method when applied for the industrial purpose.

12. This method can be applied throughout the year all over the world, since it was not affected by the weather.

13. It is assumed that the combination of pressure and "Ton platte" method will be more effective for the dehydration of agar-agar gel, and the results will be reported later.

REFERENCE

(1) Imai, T.: Japanese Patent No. 35731 (Feb., 2, 1920).

(2) Ide, M.: Japanese Patent Public Notice No. 12570 (Oct., 13, 1949).

(3) Okada, I., Osakabe, I., Kuratomi, Y., Kikuchi, T.: Journal of the Tokyo University of Fisheries, 38 (2) 291-305 (1952).

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

ON THE STUDIES OF GLUTATHION IN THE MARINE PRODUCTS
Part 1. POLAROGRAPHIC DETERMINATION OF GLUTATHION IN SEAWEEDS

Ikunosuke Okada, Isamu Osakabe, Yuji Kuratomi, Takeaki Kikuchi.

(Received on May 28, 1952)

Glutathion has been found by F. G. Hopkins[1] as tripeptide in 1921, and is contained more or less in the all parts of animals and plants. Then it is considered that glutathion plays as an important part in the biological functions. Glutathion proved to have relations with to enzymes[2·3·4·5·6], hormones[7·8·9], and vitamins[10·11·12·13·14]. According to Wachholder, Quensse[15], and Cassel[16], it is contained more in the muscles, than in all the other parts. It is considered that as glutathion has oxidizing and reducing types, it takes charge of biological oxidation and reduction in the living tissues. Then glutathion makes the biological oxidation in the living tissues possible without oxygen[17]. As the sulfohydryl-disulfide compound has great importance, the authers have tried the determination of glutathion in seaweeds by the polarographic method. The quantitative determination of the glutathion has been carried out by Ogawa[18], Fujita-Murata[19], Wachholder[20], and Katai[21]. Every method has its merit, but the polarographic method can satisfactorily be carried out in a short time, even when the other reducing substances, such as cystein and *l*-ascorbic acid, are mixed. On the polarographic studies of glutathion has reported Tachi, Kuroiwa, and Koide[22]. Go[23·24·25] has determined it polarographically in soya-bean malt, and the auther has reported the determination of it in the muscle of carp by polarography[26].

EXPERIMENTAL

Sample:—The seaweeds used in this experiment were collected at Kominato, Chiba-pref. in Oct. 10. 1951, and these species were as follows—:

Brown algae (*Phaeophyceae*)

Eisenia bicycle Setchell
Sargassum ringgoldianum Harv.
Sargassum pilubiferum C. Ag.
Padina arborescens Holwes
Sargassum tortile C. Ag.
Hijikia fusiforme Okam.
Ishige okamurai Yendo
Sargassum thunbergii O. Kuntze

Red algae (*Phodophyceae*)

Geridium amansii Lmx.
Plocamium telfairiae Harv.

Green algae (*Chlorophyceae*)
Cladophora wrightiana Harv.

Determination of Glutathion by Polarography

The current-voltage curves were recorded by means of the usual polarographic equipment using a mirror galvanometer with full sensitivity of 2.1×10^{-9} amp/mm/m at 25 ± 1°C., and a normal mercuro-sulfate electrode was used as an anode, at the room temperature. In this case, the polarogram was obtained as oxidation wave when the reducing type glutathion was oxidized, and the potencial of the oxidation wave was decided by the half wave potencial method, against saturated calomel electrode.

Standard polarogram of glutathion

Glutathion was dissolved in 5% meta-phosphoric acid, diluted with McIlvain buffer solution of pH=7.0, and concentrated to 2×10^{-4} M. Then this solution was electrolyzed, and the polarogram was obtained as shown in Fig. 1, and the

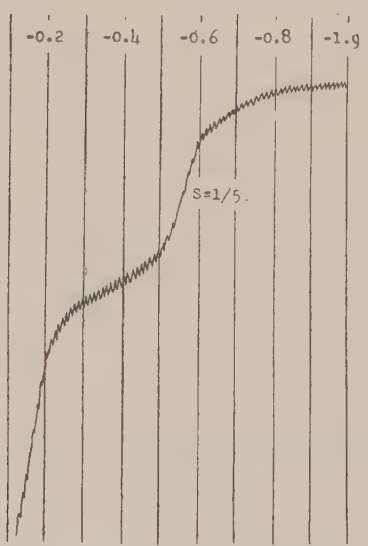


Fig. 1. Standard Polarogram of 2×10^{-4} M Glutathion in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1425 volt vs S. C. E. Anode Potencial +0.3523 volt vs S. C. E.

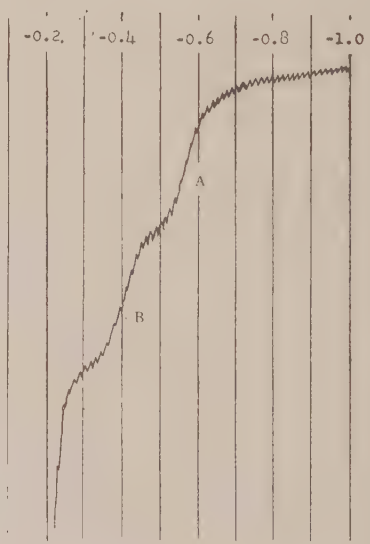


Fig. 2. Polarogram of Glutathion and Cystein in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. A) Glutathion: Oxidation potencial −0.1423 volt vs S. C. E; B) Cystein: Oxidation potencial +0.0287 volt vs S.C.E. Anode potencial +0.3757 volt vs S.C.E.

height of the oxidation wave of glutathion was 37.7 mm (sens. of Galv. =1/5), and the oxidation potencial was −0.1425 volt vs S. C. E. In this experiment, the glutathion with the purity of 99% was used. In this case, when the reducing substances such as *l*-ascorbic acid, cystein were contained, the oxidation waves also appeared. According to polarographic studies of *l*-ascorbic acid by Tachi, Okada[27·28], Go[25], the oxidation potencial of *l*-ascorbic acid was −0.055 volt vs S. C. E. (pH=7.0), and the potencial of glutathion was −0.142 volt vs S. C. E. (pH=7.0), therefore, glutathion and *l*-ascorbic acid can be determined separately on the polarogram by the difference of oxidation potencials. When glutathion and cystein existed, as shown in Fig. 2., the oxidation potencial of glutathion was −0.142 volt vs S. C. E. (pH=7.0) and that of cystein was +0.029 volt vs S. C. E. (pH=7.0), so that both substances can satisfactorily be separated.

Determination of glutathion in seaweeds

"*Eisenia bicycle* Setchell" was cut into small pieces, and 5 g of the cut sample was crushed with a small quantity of quartz sand, and stirred with 10 cc. of 5% meta-phosphoric acid solution and after filtered, McIlvain buffer solution of pH=7.0 (equal to the filtrate in quantity) was added to the filtrate. The above mentioned extracted solution was electrolysed by the polarographic method, and polarogram was obtained as shown in fig. 3. The height of the oxidation wave of glutathion was 13.0 mm (Sens. of Galv. =1/10), therefore, 22.6 mg% of the glutathion was contained in this "*Eisenia bicycle* Setchell" as compared with the standard glutathion. The content of moisture in this sample was 70.6%. The above mentioned polarographic determination of glutathion was applied to the other seaweeds and results were shown in the next table, and the polarograms (Fig. 4∼13). According to the results as shown on the Fig. 3, "*Eisenia bicycle* Setchell" and Fig. 8, "*Ishige okamurai* Yendo", there is obtained an oxidation wave besides that of glutathion. It may be assumed that the oxidation waves (−0.0361 volt, Fig. 3) and (−0.0928 volt, Fig. 8) is *l*-ascorbic acid.

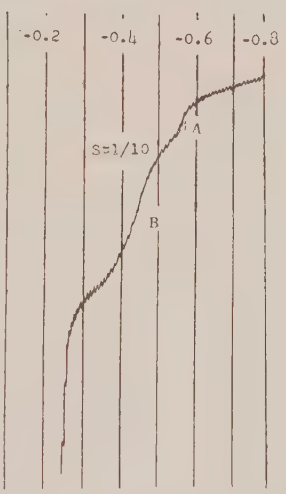


Fig. 3. Polarogram of "*Eisenia bicycle* Setchell" in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. A) Oxidation potencial −0.1531 volt vs S. C. E; B) Oxidation potencial −0.0361 volt vs S. C. E; Anode potencial +0.4019 volt vs S. C. E.

The contents of glutathion in seaweeds by polarography

Sample	Polarogram	Solution	Oxidation potencial	Height of wave	Sensitivity of galvanometer	Contents of glutathion	Moisture of sample
			volt	mm		mg%	%
Standard glutathion	Fig. 1	2×10^{-4} *pH=7.0	−0.1425	37.7	1/5	6.14	
Eisenia bicycle Setchell	Fig. 3	Sample 5g 5%HPO_3 10cc *pH=7.0	−0.1531	13.0	1/10	22.62	70.6
Sargassum tortile C. Ag.	Fig. 4	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1398	15.0	1/5	19.35	72.4
Sargassum ringgoldianum Harv.	Fig. 5	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1505	16.0	1/5	20.46	63.3
Hijikia fusiforme Okam.	Fig. 6	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1383	13.0	1/5	13.87	76.6
Sargassum piluliferum C. Ag.	Fig. 7	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1333	23.5	1/10	60.60	70.6
Ishige okamurai Yendo	Fig. 8	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1257	3.5	1/10	8.87	62.3
Padina arborescens Holmes	Fig. 9	Sample 5g 5%HPO_3 10cc *pH=7.0	−0.1488	8.0	1/5	6.59	56.0
Sargassum thunbergii O. Kuntze	Fig. 10	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1499	19.5	1/5	25.14	69.3
Gelidium amanssii Lmx.	Fig. 11	Sample 5g 5%HPO_3 10cc *pH=7.0	−0.1483	18.0	1/5	15.66	70.6
Plocamium telfairiae Harv.	Fig. 12	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1513	3.0	1/5	3.87	70.5
Cladophora wrightiana Harv.	Fig. 13	Sample 3g 5%HPO_3 10cc *pH=7.0	−0.1405	10.0	1/2	5.22	71.7

Note: *: McIlvain buffer solution of pH=7.0 was used, and it volume was equal to the 5% HPO_3 solution extracts.

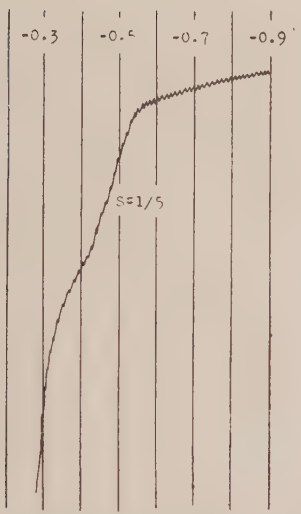


Fig. 4. Polarogram of "*Sargassum tortile* C. AG." (in McIlvain buffer of pH =7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1398 volt vs S. C. E. Anode potencial +0.3352 volt vs S. C. E.

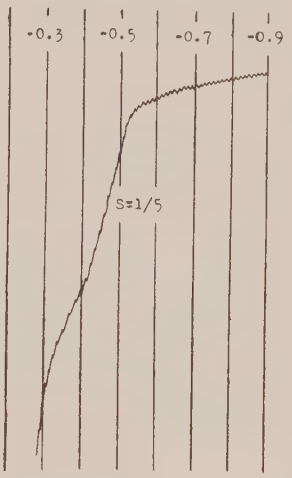


Fig. 5. Polarogram of "*Sargassum ringgoldianum* HARV." in McIlvain buffer pH=7.0 using a normal mercurosulfate electrode. Oxidation potencial −0.1505 volt vs S. C. E. Anode potencial +0.3495 volt vs S. C. E.

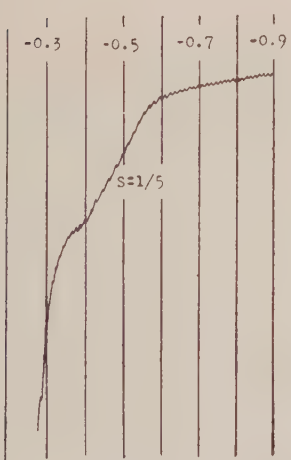


Fig. 6. Polarogram of "*Hijikia fusiforme* OKAM." in McIlvain buffer of pH =7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1383 volt vs S. C. E. Anode potencial +0.3467 volt vs S. C. E.

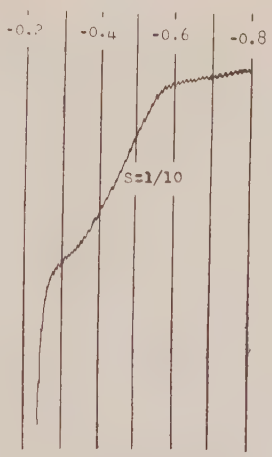


Fig. 7. Polarogram of "*Sargassum piluliferum* C. AG." in McIlvain buffer pH =7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1333 volt vs S. C. E. Anode potencial +0.3417 volt vs S. C. E.

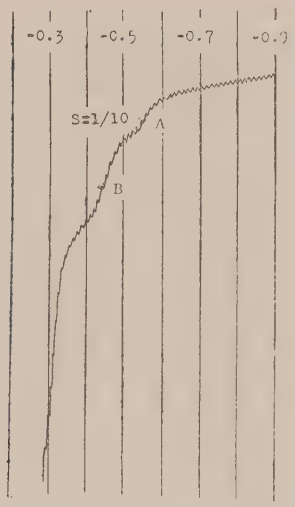


Fig. 8. Polarogram of "*Ishige okamurai* Yendo" in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. A) Oxidation potencial −0.1257 volt vs S. C. E. B) Oxidation potencial −0.0298 volt vs S. C. E. Anode Potencial +0.3372 volt vs S. C. E.

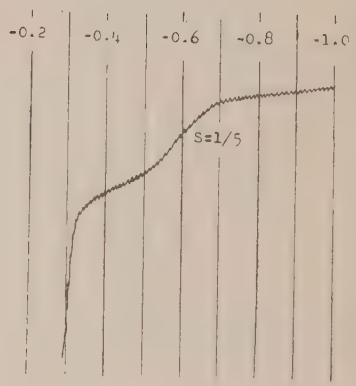


Fig. 9. Polarogram of "*Padina arborescens* Holmes" in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1488 volt vs S. C. E. Anode potencial +0.4412 volt vs S. C. E.

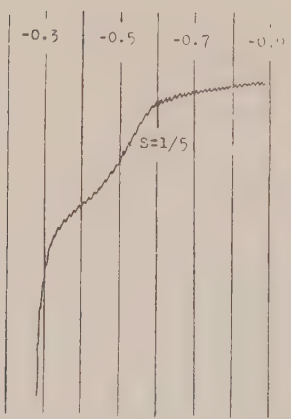


Fig. 10. Polarogram of "*Sargassum thunbergii* O. Kuntze" in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1499 volt vs S. C. E. Anode potencial +0.3637 volt vs S. C. E.

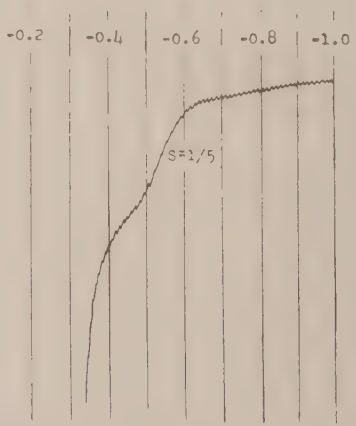


Fig. 11. Polarogram of "*Gelidium amanssii* Lmx." in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1483 volt vs S. C. E. Anode potencial +0.4767 volt vs S. C. E.

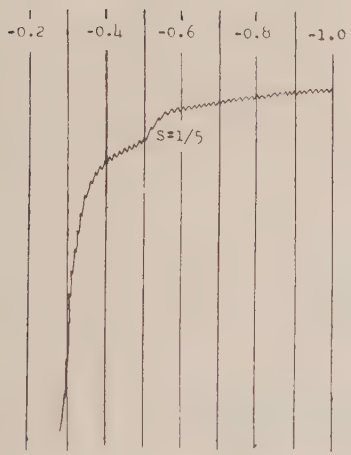


Fig. 12. Polarogram of "*Plocamium telfairiae* HARV." in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1513 volt vs S. C. E. Anode potencial +0.3637 volt vs S. C. E.

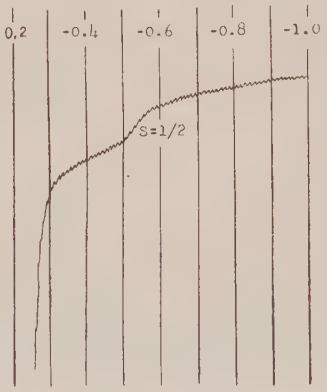


Fig. 13. Polarogram of "*Cladophora wrightiana* HARV." in McIlvain buffer of pH=7.0 using a normal mercuro-sulfate electrode. Oxidation potencial −0.1405 volt vs S. C. E. Anode potencial +0.3375 volt vs S. C. E.

Résumé

1) The polarogram is the experiment that indicated the reduced type of glutathion.

2) In the polarographic determination of glutathion, it is easily accomplished in a short time, even when the other reducing substances, such as *l*-ascorbic acid and cystein exist.

3) Glutathion was contained more or less in the seaweeds in this experiment, the contents of glutathion were almost 10∼20 mg%, the maximum content of it was 60.60 mg % in "*Sargassum piluliferum* HARV.", and the minimum content of it was 3.87 mg% in "*Plocamium telfairiae* HARV.". There is no difference in the content of glutathion among "*Phacophyceae*", "*Rhodophyceae*", and "*Chlorophyceae*". Though the content of glutathion in the seaweeds varies according to seasons, we get the above mentioned results from the contents of the samples collected in Oct. 10, 1951.

4) As glutathion was contained more or less in seaweeds, it may safely be assumed that it takes charge of the molecular respiration in the living tissue.

5) It has a deep significance that glutathion is contained in the seaweeds, because the sulfohydryl-disulfide compound is important for biological function.

Finally, we wish to express our hearty thanks to Prof. Tachi of the Kyoto Univ., who helped us in this experiment, and also we cannot forget to thank Mr. Umeda and Kuroiwa, ("Kirin" Scientific Research Institute) for their kind offer of pure glutathion. We wish also to record our thanks for the financial aid given by the Ministry of Education for pursuing the present study.

REFERENCE

1) Hopkins, F. G.: Biochem. J. 15, 286 (1921).
2) Lohmann, K.: Biochem. Z. 237, 445 (1931).
3) Lohmann, K.: Ebenda 237, 447 (1931).
4) Lohmann, K.: Ebenda 254, 332 (1932).
5) Meyerhof, O., Lohmann, K.: Nature 20, 389 (1932).
6) Meyer, T., Borger, G.: Biochem. Z. 273, 56 (1934).
7) Kawauchi: (J. Med. Soc. Mari., in Jap.) 53, 435 (1934).
8) Ito: (J. Pharm. Soc. Jap., in Jap.) 33, 53 (1941).
9) Binet, L., Weller, G.: Bull. Biol. Pharm., 233 (1938).
10) Abe: (J. Med. Soc. Hokkaido, in Jap.) 14, 1207 (1936).
11) Rondoin, L., Farbe, R.: Bull. Soc. Chim. Biol. 9, 1027 (1927).
12) Hopkins, F. G., Morgan, E. J.: Biochem. J., 30, 1446 (1936).
13) Kertesty, Z. I.: Biochem, J., 32, 621 (1938).
14) Fujita, A., Numata, S.: Biochem. Z., 299, 249 (1938).
15) Wachholder, K., Quensee, W.: Arch. Ges. Physiol., 235, 70 (1934).
16) Cassel, L.: Arch. Ges. Physiol., 235, 30 (1935).
17) Mori: p. 99. (The Theory and Means of Organic Chemistry., in Jap.)
18) Ogawa: J. Agr. Chem. Soc. Jap., 14, 65 (1938).
19) Fujita, Numata: The Tokyo Med. J., 3092-1, 3093-1.
20) Wachholder, K., Unrenbrook, K.: Z. Physiol. Chem., 233, 181 (1935).
21) Katai: J. Agr. Chem. Soc. Jap., 18, 379 (1942).
22) Tachi, Kuroiwa, Koide: Express to the Japan Vitamin Society, May. 28, 1949.
23) Go: J. Ferm. Tech. Jap., 28, 79 (1950).
24) Go: J. Ferm. Tech. Jap., 28, 110 (1950).
25) Go: J. Ferm. Tech. Jap., 28, 178 (1950).
26) Okada, Kuratomi: Express to the Japanese Society of Scientific Fisheries May 6, 1951.
27) Okada: J. Agr. Chem. Soc. Jap., 19, 749. 955 (1943).
28) Okada: J. Agr. Chem. Soc. Jap., 20, 325 (1944).

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

ON THE EFFICACY OF NET PRESERVATIVES
Part. I. ABOUT SOME AROMATIC CHLORINE DERIVATIVES

Ikunosuke Okada, Katuji Honda, Kenzo Toyama, Takeaki Kikuchi

(Received on May 28, 1952)

The authors studied on fish net preservatives about some aromatic chlorine derivatives that is pentachlorophenol, santobrite (sodium pentachlorophenate), benzylchloride and o-chlorotoluene.

Pentachlorophenol and santobrite are well known to be an exceedingly toxic substance to lower plant life, bacteria, algae etc. and highly antagonistic to certain ferments, and therefore seemed a good respect for trial in different forms.

For fish-net preservative K. Kanna was[1] reported, as a result of experimental exposure, that P.C.P. (pentachlorophenol) and santobrite treated line showed little superiority to untreated line.

The authors tried to convert in a insoluble form of santobrite and fixing on net-cord by $CuSO_4$ solution treating.

This trial get a successful results.

EXPERIMENTAL

(1) Notation of sample.

In the following key all samples designated by the same letter of the Alphabet are always treated with the same preservatives in the same way. In all the results recorded herein the symbols and identifying number refer to this key.

Key to symbols for preservatives studied

A ....White line-untreated—cotton line (20'S $^3/_{15}$)—used as a control.

B ....Cutch ($K_2Cr_2O_7 \cdot CuSO_4$)–2%cutch concentration in water. Lines were dipped into this solution and allowed to become through saturated. This required 30 minuts at boiling point. Then the lines were treated with 1% $K_2Cr_2O_7$ and 3% $CuSO_4$ mixture 30 minuts at 80°C and immediatly washed in water and spread to dry (not piled in a heap)—used as a control.

C ....Coal tar—The lines were soaked in heavy tar (not dilute with creosote) at 80°C. 30 minuts.

As the lines were drawn out of the tar they were drawn between the

fingers in order to remove the excess tar adhering to the lines—used as a control.

D .....P. C. P.—5% concentration in benzol—Lines were dipped into this solution and allowed to become through saturated (no heating was necessary)

E .....P. C. P.+Coal tar—The first treating was as same as D, and second coal tar was used. Treated as C.

F.....P. C. P. (+soapless soap)—This was the same as D, but containing a few of soapless soap owing to good penetration of P. C. P.

G .....Santobrite + $CuSO_4$—The first 5% santobrite conc. in water was used. It required 60 minuts at 70°C, to saturated in this solution. Second 1% $CuSO_4$ solution was used and soaked in this solution 30 minuts at 70°C.

H.....Santobrite + $ZnCl_2$—The first treating was as same as G, and second 1% $ZnCl_2$ solution was used instead of 1% $CuSO_4$ solution.

I .....Santobrite + $CuSO_4$ + Coal Tar—The first and second bath was as same as G, and the third bath was used heavy tar as C.

J.....Benzyl chloride—This is an chlorine derivative of toluene in side chaine and it was a liquid having a characteristic aromatic odour. The vapour is irritating to the eyes, having a specific gravity D_{15}^{15} 1.104, and boiling point of 179°C. This applied by allowing the line to soak in this liquid for 30 minuts at 70°C.

K.....O—chlorotoluene—This is an chlorine derivative of toluene in the neucleus. Its specific gravity was D_{15}^{15} 1.0749, and boiling point was 159°C. In this solution the line was treated 30 minuts at 70°C.

(2) Material and method.

Cotton lines (20′S $^3/_{15}$) were prepared by each of 4 preservatives, namely P. C. P. in 3 different treatment D, E, and F; santobrite in 3 different combinations, G, H, and I, Benzyl chloride, J; and o—chlorotoluene, K. And untreated white line A, cutch B, and coal tar C, were used as a control. 10 samples were prepared by each preservative, one to hold as an unexposed check; the other 9 to be exposed.

These samples were fastend to a iron frame and immersed in sea water at Tateyama Bay (Chiba Prefecture) from July 23 to Oct. 21, 1951.

At every ten days one sample treated by each preservative and 3 control samples were removed and observed marine growth on the lines and then tested for tensile strength and elongation, as it was in wet condition.

(3) Water condition at Tateyama.

The temperature and density of water during the period the lines were exposed were shown in Table I.

Thre were abundant growth of barnacless Bryozoa, and othe attached marine growths.

Table 1. Water condition at Tateyama.

Period	Water temperature	Density (15°C.)
July. 23—Aug. 2	24.2 °C	24.72
Aug. 3—Aug. 12	26.0	24.93
Aug. 13—Aug. 22	26.1	24.71
Aug. 23—Sep. 1	26.8	24.63
Sep. 2—Sep. 11	24.2	24.50
Sep. 12—Sep. 21	23.9	23.45
Sep. 22—Oct. 1	24.8	24.15
Oct. 2—Oct. 11	22.2	23.95
Oct. 12—Oct. 21	23.1	24.70

Table 2. Tensile strength of cotton lines exposod in sea water at Tateyama.

Symbol and treatment	Number of days exposed								
	unexposed	10	20	30	40	50	60	70	80	90
	Tensile strength in Kg.									
A-White line, untreated control	6.45	6.68	5.44	3.20	—	—	—	—	—	—
B-Cutch 2% ($K_2Cr_2O_7$. $CuSO_4$) control	7.07	7.11	6.84	6.47	5.40	3.97	3.00	—	—	—
C-Coal tar, control	5.50	5.14	5.01	4.83	5.03	4.49	4.89	3.84	3.81	—
D-P.C.P. 5% in benzol	7.58	7.87	6.21	1.63	—	—	—	—	—	—
E-P.C.P.+Coaltar	5.01	—	4.83	5.59	4.99	5.41	5.06	4.34	4.44	3.74
F-P.C.P. (+soapless soap)	7.27	7.85	5.40	2.79	1.90	—	—	—	—	—
G-Santobrite 5% +$CuSO_4$	7.25	7.18	6.36	5.78	5.27	3.65	2.36	—	—	—
H-Santobrite + $ZnCl_2$	6.53	6.81	5.84	2.37	2.19	—	—	—	—	—
I-Santobrite + $CuSO_4$ + Coal tar	5.40	5.11	5.04	5.13	5.36	5.31	5.21	5.34	4.81	4.40
J-Benzyl chloride	6.59	7.01	6.23	5.81	3.19	1.39	—	—	—	—
K-O-chlorotoluene	6.51	6.86	5.78	3.90	2.79	—	—	—	—	—

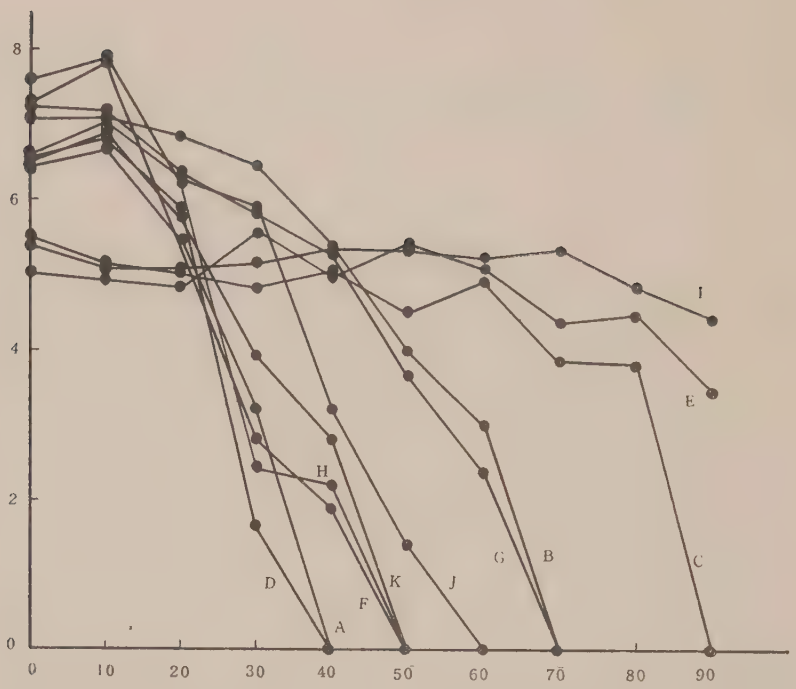


Fig. 1. Tensile strength of cotton line exposed in sea water at Tateyama. Ordinate.....tensile strength. (Kg.); Abscissa.....days exposed.

Table 3. Elongation of cotton lines exposed in sea water at Tateyama.

Symbol and treatment	unexposed	Number of days exposed								
		10	20	30	40	50	60	70	80	90
	Elongation %									
A-White line, untreated control	21.7	18.5	18.3	12.5	—	—	—	—	—	—
B-Cutch 2% ($K_2Cr_2O_7$. $CuSO_4$) control	19.2	19.8	22.5	19.9	18.7	17.5	14.0	—	—	—
C-Coal tar, control	20.8	22.9	23.9	26.1	20.1	17.5	16.2	12.1	13.2	—
D-P.C.P. 5% in Benzol	20.3	18.6	19.7	14.3	—	—	—	—	—	—
E-P.C.P. + Coal tar	20.5	—	22.4	18.8	17.6	14.4	16.1	12.8	15.1	12.8
F-P.C.P. (+ soapless soap)	20.1	17.4	19.1	14.1	9.6	—	—	—	—	—
G-Santobrite 5% + $CuSO_4$	22.6	20.0	20.8	19.8	17.1	17.2	13.5	—	—	—
H-Santobrite 5% + $ZnCl_2$	21.1	20.0	22.3	15.1	14.8	—	—	—	—	—
I-Santobrite + $CuSO_4$ + Coaltar	18.5	20.9	19.9	22.8	16.9	16.9	16.6	15.5	16.2	13.7
J-Benzyl chloride	18.3	19.9	22.5	15.0	14.7	10.3	—	—	—	—
K-O-chlorotoluene	19.4	20.9	21.0	14.1	14.5	—	—	—	—	—

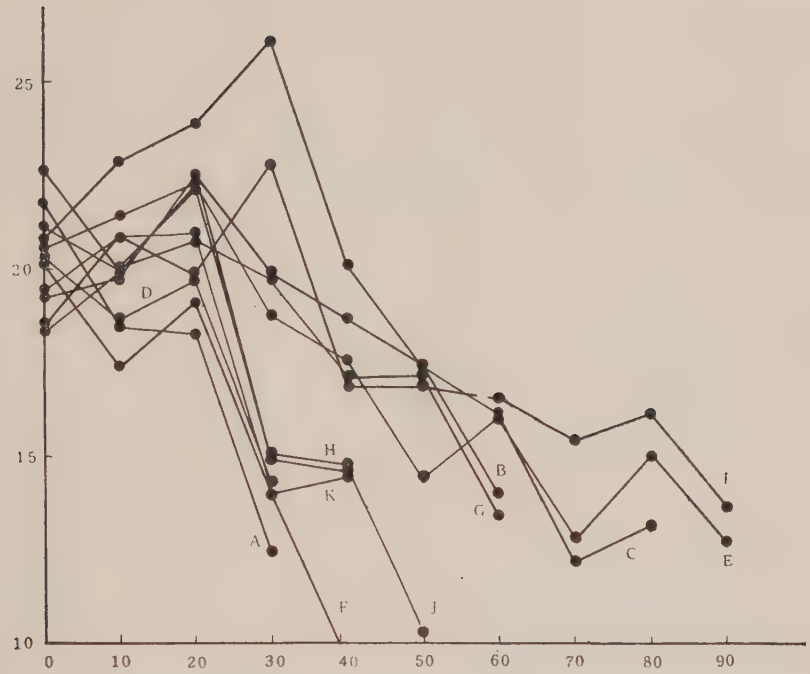


Fig. 2. Elongation of cotton line exposed in sea water at Tateyama.
Ordinate.....elongation (%); Abscissa.....days exposed.

(4) Tensile strength, elongation and effective day.

Because of variation in the line it is necessary to take the avarage of a number of tests. The figure given for these samples were avarage of 10 tests in wet condition. Table 2 gives the results of the measurment of tensile strength. The same results were expressed graphically in Fig. 1. And elongation were shown in Table 3, and graphically in Fig. 2.

According to M. Tauchi's study[2]; when a net cord is exposed in water, its original tensile strength is maintained for some days, as yet there is no rotteness, after which a very rapid deterioration occur.

And the relation was represented as follows:—

$$\log T/(T_0-T)=-k(\log e)t+C \qquad (1)$$

Where;

T_0=Tensile strength of line before a rapid deterioration.
T =Tensile strength of line after t days.
t =Number of days exposed.
k and C=constant (k=weaken coefficient).

where: $t_e = t_0 + t_\tau$ (2)

t_e = Effective days of preservation

t_0 = Days that the line is maintained for its original tensile strength.

t_τ = Days from a rapid detrioration to the lines tensile strength reduce by one half* of its original respectively. (calculated by formula (I))

The results calculated by M. Tauchi's equation are shown in Table 4.

Table 4. Effective period of cotton lines exposed in sea water at Tateyama.

Symbol and treatment	t_0	t_e	$k \times 10^2$
A. untreated.	10	30	7.2
B. cutch ($K_2Cr_2O_7.CuSO_4$)	10	52	4.5
C. Coal tar	0	98	1.4
D. P.C.P. benzol solution	—	—	—
E. P.C.P. + coal tar	50	105	2.4
F. P.C.P. (+ soapless soap)	10	27	4.5
G. Santobrite + $CuSO_4$	10	52	2.9
H. Santobrite + $ZnCl_2$	10	31	7.0
I. Santobrite + $CuSO_4$ + Coal tar	—	—	—
J. Benzyl chloride	10	38	5.0
K. O-chlorotoluene	10	37	4.5

(5) Results.

1) P.C.P. treated only, D; was a poor results, that is similar to Kanna's report, and with soapless soap, owing to a good penetration of P.C.P., F; was worthless too. But P.C.P. with coal tar, E; showed little superiority tocoal tar, C. It's original tensilestrength was mainteined after 50 days and effective days was 105 days, and coal tar used as a control was 98 days, and from a view-point of weaken coefficient it is inferior to coaltar. (Table 4)

2) Santobrite followed by a treatment with $CuSO_4$, G; was effective like cutch treated with $CuSO_4$ and $K_2Cr_2O_7$, B.

It's effective day was 52 days and cutch used as a control was the same one, and weaken coefficient of it was 0.029 and cutch was 0.045 (Table 4). It showed little superiority of weaken coefficient to cutch treated.

* In this case the value showed one half of original tensile strength of untreated line.

But santobrite with $ZnCl_2$ instead of $CuSO_4$ treated, H; showed no favorable effect. It's effective day was 31 days and weaken coefficient was 0.070 (Table 4). Santobrite (with $CuSO_4$) with coal tar, I; preserved fairly well compared with coal tar. In this case it was difficult to calculate of this effective day. But its original tensile strength was kept after 70 days, and after 90 days tensile strangth was 4.40 Kg. that the figure was larger than one half of original tensile strength of untreated line (Fig. 1). It seemed that some santobrite deposited in the line with coal tar and preserved well.

3) Benzyl chloide, J; and O-chlorotoluene, K; were showed little superiority to untreated line. The former was 38 days on effective day and 0.050 on weaken coefficient and the latter was 37 days and 0.045 respectively (Table 4). Therefore there is no difference on the efficacy of net preservatives between side chain chlorine derivative Benzylchloride and neucleous chlorine derivative O-chlorotoluene.

(6) Resistance to fouling by marine growth.

When net remain in water for long time, as pound nets traps, etc, must they are subject to fouling by growth by barnacles, ascidians, algae, etc. Barnacles injure the fishermans hands and make handling the net difficult fouling growth cause the resist current and tides, which accordingly causes greater strains on the nets. The weight of the net and therefore greatly increase the strain on it. It is a desirable characteristic of net preservatives that they prevent the accumulation of these growth.

On this report give an outline of the condition after two month.

The condition of lines after two month exposed in sea water at Tateyama.

Symbol and treatment	Condition after two months in water
A—White line; no treatment	Slightly fouled some algae; no barnacles.
B—Cutch 2% ($K_2Cr_2O_7.CuSO_4$)	Do.
C—Coal tar	Covered with many of barnacles.
D—P.C.P. 5% in benzol	Few algae and barnacles; nearly clean.
E—P.C.P.+Coal tar	Fouled, less than coal tar; C.
F—P.C.P. (+soapless soap)	Few algae and barnacles; nealy clean.
G—Santobrite 5%+$CuSO_4$	Substantially clean; very few algae and barnacles.
H—Santobrite 5%+$ZnCl_2$.	Do.
I—Santobrite+$CuSO_4$+Coal tar. . . .	Fouled with some barnacles, less than coal tar C.
J—Benzylchloride	Slightly fouled; some algae and barnacles.
K—O-chlorotoluene	Do.

SUMMARY

The principal resuls and conclusions arrived at and report in detail in the main part of this paper are here summarized.

1) The following preservatives were tested; pentachlorophenol, santobrite (sold under a trade name) that is sodium penta chlorophenate, benzyl chloride and o-chlorotoluene.

2) The textiles tested were cotton line (20'S $^{3}/_{15}$).

3) The factors determined were tensile strength, elongation, effective day, and resistance to fouling by marine growth.

4) Series of lines treated with various preservatives were exposed in Tateyama Bay (Chiba Prefecture), from July 23 to Oct. 21, 1951.

5) P.C.P. was a poor preservative. Line treated only by this showed no superiority to untreated line.

6) Santobrite followed by a treatment with $CuSO_4$ was almost like cutch treated with $CuSO_4$ and $K_2Cr_2O_7$. It seemed that converting method in insoluble form of santobrite by $CuSO_4$ treating was effective.

7) Santobrite with $ZnCl_2$ instead of $CuSO_4$ showed no favorable effect. Line treated by this showed almost the same to untreated.

8) Santobrite (with $CuSO_4$) or P.C.P. followed by a treatment with coal tar were better than coal tar. It seems that some santobrite and P.C.P. deposited in the line and preserved well.

9) Benzyl chloride and o-chlorotoluene were showed little superiority to untreated line. In this case, there is no difference of preservative effect between side chaine chlorine derivative Benzyl chloride and neucleous chlorine derivative O-chlorotoluene.

Finally, the authors to express their hearty thanks to Mr. M. Ishino, who has assisted this work, and wish to express their thanks to the Ministry of Education by which the present investigation has undergone the financial aid.

REFERANCE

(1) Kanna, K. 1951 : Presented at the Annual meeting (at Maizuru) of the Japanese Society of Scientific Fisheries.

(2) Tauchi, M. 1927 : Jour. Imp. Fish. Inst. 23, 1.

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

EFFECT OF POPULATION-DENSIYY AND POND-AREA ON THE GROWTH OF FISH

Tomokichi Yoshihara

(Received on Sept. 30, 1952)

1. INTRODUCTION

There are many investigations on the relation between the length and the weight of fish, while unfortunately available data offer no basis on the effect of population-density and pond-area.

The problems of effects of condition of environment on the growth rate of fish can be approached more effectively through the understanding of principles revealed during breeding experiments than through the observations under natural conditions, since it is difficult to get favourable uniform conditions in nature. But the experimental results are rare because the study of growth by the breeding experiment during the long period is difficult due principally to its considerable requirement of cost, space and time.

The physiological mechanism of the growth of fish still remains very obscure: but there are some interesting hints and possiblities to demonstrate the growth by the logistic curve, because this curve is derived through a theoretical ground and may be applied to the individual growth, as well as the increase of number of individuals of population, e.g. the growth of body length or incrcase of body weight.

The following report of the experiments is presented in order to deepen our understandings of density-area-effect on the growth of fish population and to point out the validity of logistic curve when applied to the individual growth. At the same time the changes of the condition factor of the groups reared under different conditions of enviroment and the influence of population density upon the rate of reproduction are examined.

It is my greatest pleasure to express my hearty thanks to Mr. S. Shimizu, Director of Gumma Fish Culture Experiment Station, where this experiment was done, for his kindness and the financial helps. A part of the studies was carried out through the Goverment Grant to Fundamental Scientific Research.

2. MATERIALS

The Carp *Cyprinus carpio* Linné, was used. This fish is one of the commonest and widely cultured fresh water fish in Japan and appropriate to

this experiment as it is easy to culture. The investigations of growth of this fish attract attentions because it has economic importance. Works on the breeding have been done by many workers but very little information has been

Table 1. The growth of carp

No. of pond		I	II	III	IV	V	VI	VII	VIII	IX
Total length (mm.)	1950 May 18	168	156	159	145	151	157	153	146	138
	July 7	203	200	217	202	193	193	193	185	171
	Aug. 26	274	253	279	239	221	238	236	215	203
	Oct. 15	135	318	313	260	245	270	257	231	220
	△	+17	+10	+7	+17	+26	+5	+10	+16	+10
	1951 June 13	332	328	320	277	271	275	267	247	230
	Aug. 1	365	367	361	320	302	306	300	278	258
	Sept. 20	401	409	395	351	336	339	332	306	285
	Nov. 9	413	420	406	358	340	334	328	305	293
	△	—	—	−7	+8	0	+22	+16	+9	−4
	1952 May 27	—	—	399	366	339	356	346	314	289
Body weight (g.)	1950 May 18	79	62	81	76	61	68	73	65	50
	July 7	138	145	177	126	116	127	132	110	91
	Aug. 26	328	313	374	238	194	264	218	178	154
	Oct. 15	495	568	524	318	270	371	304	218	208
	△	−38	−50	−56	−6	+12	−73	−35	−1	−31
	1951 June 13	457	518	468	312	282	298	269	217	177
	Aug. 1	885	908	762	613	475	426	479	357	285
	Sept. 20	1150	1264	1162	793	695	696	683	499	443
	Nov. 9	1163	1313	1158	860	700	700	681	480	451
	△	—	—	+8	+17	−57	−74	+3	−49	−50
	1952 May 27	—	—	11	878	643	626	684	431	401
Group of pond		A	A	A	B	B	B	C	C	C
Number of fish		2	6	18	4	12	36	8	24	72
Area of pond (square meter)		3.91	11.82	36.00	3.91	11.82	36.00	3.91	11.82	36.00
Density		0.51	0.51	0.51	1.02	1.02	1.02	2.04	2.04	2.04
Values of n		2.98	2.74	2.74	2.92	3.08	2.84	2.80	2.68	3.14
Values of b		2.21	2.12	1.82	2.24	2.09	2.36	2.09	2.14	2.13

△ denote the increase (+) or decrease (−) of the total length or body weight during winter seasons.
Figures in this table are the mean values and variations are omitted.

furnished on its growth under different conditions of population density, with which the present paper deals.

Nine small ponds were made by dividing each of three ponds A, B and C of the same area, into three parts in the ratio of 1: 3: 9, then stocked with different numbers of fish on May 18, 1950. Thus in each pond of the groups A, B and C, the population density of fish is different as shown in Table 1. The depth of pond water is about 60 cm. For details of data see Table 1.

Diets were given every day except the days of bad weather and winter season as shown in Table 2. Chemical compositions of diets are given in Table 3.

Table 2. Weight of diets during each peroid of experiments.

Period		1950			1950–1951	1951			1951–1952
		May 18 – July 6	July 7 – Aug. 25	Aug. 26 – Oct. 15	Winter season	June 13 – Aug. 1	Aug. 1 – Sept. 21	Sept. 22 – Nov. 9	Winter season
Water temperature (C°)		18-25	23-28	21-27	—	19-28	20-28	—	—
Weight (Kg.)	Isaza	40.34	—	—	—	—	—	—	—
	Sanagi	27.72	58.34	38.38	7.63	74.82	78.73	35.41	—
	Saibaku	61.28	44.21	31.83	—	—	—	—	—
	Omugi	—	—	—		30.80	34.59	35.41	—
	Total	129.34	102.55	70.21	7.63	105.62	113.24	70.82	—
Composition (Kg.)	Moisture	15.25	8.91	6.28	0.61	9.71	10.52	9.15	—
	Protein	44.02	38.63	25.72	4.48	48.05	50.91	25.51	—
	Fat	13.13	17.40	11.50	2.14	21.45	21.72	10.54	—
	Ash	11.18	3.79	2.61	0.23	2.74	2.95	1.66	—
	Tissue	5.15	3.71	2.68	—	1.69	1.90	1.95	—
	Sol. non-*N* substance	39.70	28.60	20.60	—	20.15	22.63	23.20	—
	Total	127.29	101.34	69.39	7.46	103.29	110.63	70.01	—

Table 3. Chemical compositions of diets (per mille).

		Moisture	Protein	Fat	Ash	Tissue	Sol. non-*N* substance	Total
Isaza	Mysidacea (Fairy shrimp)	170	583	95	187	—	—	970
Sanagi	Silkworm pupa	80	588	280	30	—	—	978
Saibaku	Barley	101	98	25	46	84	646	1000
Omugi	Barley	122	133	18	17	55	656	1000

3. METHOD OF MEASUREMENT

At intervals of fifty days the fish of each pond were removed by a small net to a container, then measured to the nearest millimeter and weighed to the nearest gram but as the fish grow the precision of the measurements were changed to five millimeters and five grams, because it was impossible to take accurate measurements. Results are shown in Table 1 and graphs of the

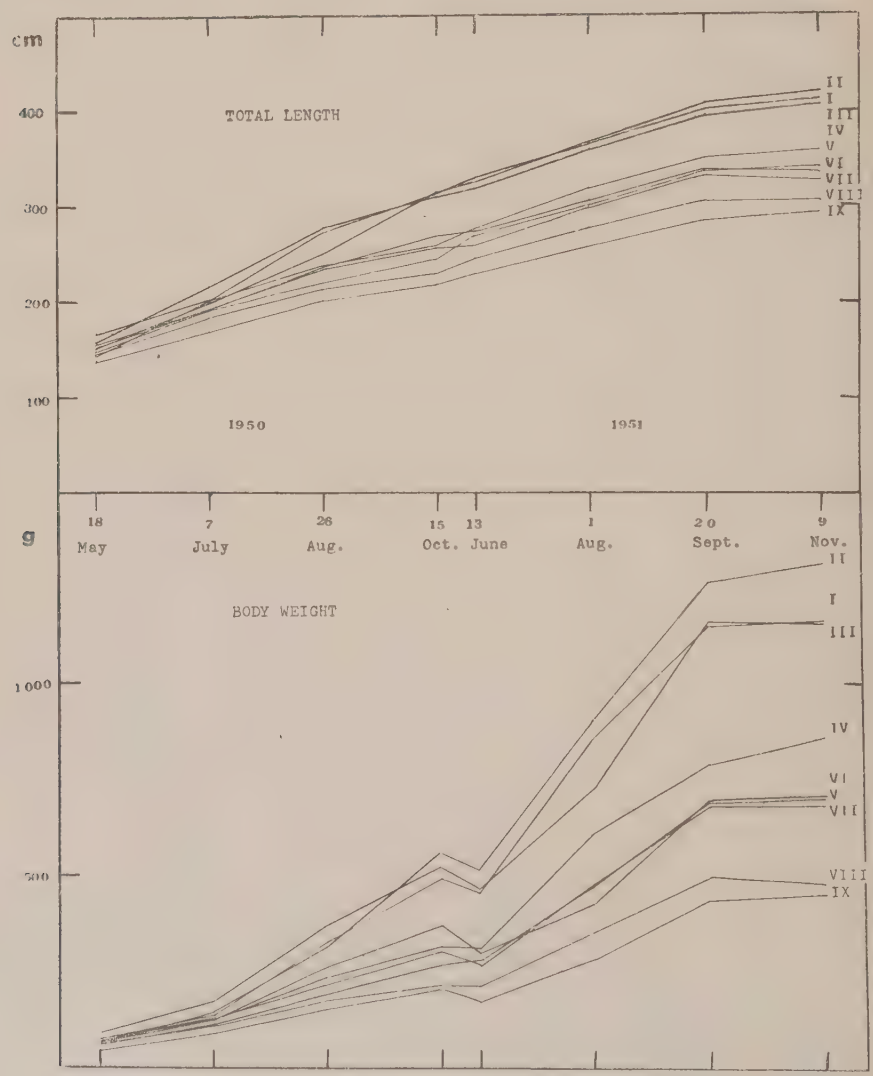


Fig. 1. Comaparision of the growth of the total length and the body weight of fish in each pond. Figures in Roman type show the number of pond.

increase of length and weight of fish in each pond are shown in Fig. 1. Measurement was taken for each individual but the growth of fish is represented by the mean growth curve because the marking of individuals was unsuccessful.

Carps of one year old were used in order to avoid high mortality during the experiments. Consequently the number of dead fish resulting from rearing, handiang and disease was small and the effect of them may be ignored. The decrease in number of fish during the experiment by various reasons were supplemented on the day of successive measurements. At the beginning of the experiment the length and weight of fish of each pond were not same but the errors caused by these irregularities were ignored.

In 1951, the commencement of the experiment was delayed. Thus at the last measurement the weight of fish decreased, since in November fish ceased to feed diets. On the other hand the length of fish did not changed regularly, therefore only the results of measurements on weight during 1950 were used for discussion.

4. RESULTS

(i) Trends of the growth curve and change of values of the condition factor .

The results obtained from this experiment suggest that, as a first approximation, the growth of fiish may be represented by a straight line in each year and the effects of density of population may be shown by the slope of the line of Fig. 1.

In winter the weight of fish decreased but the length increased. This phenomenon was confirmed by the examination of the values of the condition factor, which is defined by

$$F=1000\,W/L^3$$

where W in gram and L in cm. In the first year the values of F decreased generally during the summer when fish grow rapidly. There are considerable changes in the values of F within the year. At the same the similar phenomena are observed under natural conditions. Details of the results will be shown in the succeeding paper.[7] Sometimes the values of the condition factor are defined by

$$F=1000\,W/L^n$$

Values of n were determined and are shown in Table 1.

(ii) An experimental veriffication for the validity of the logistic curve.

If the population increases freely, then it follows that the total population size $y(x)$ must satisfy a differential equation of the form

$$dy/dx=by$$

where b is the intrinsic rate of growth *per capita* per unit of time, x time and y number of individuals of the population. The solution, when b is constant, is, of course,

$$y(x)=y_0 \ exp \ bx$$

the law of Malthus.

If now the hypothesis that b falls off linearly with an increase in the total population size, because of overcrowding, for example, is assumed then the differential equation takes on the form

$$dy/dx=by-hy^2 \tag{1}$$

with the solution

$$y=\frac{L}{1+exp\ (a-bx)} \tag{2}$$

the logistic law of Verhulst. In equation (2)

a=a constant concerning the origin of time,
b=the intrinsic rate of natural inerease or the biotic potential,
h=the limiting coefficient,
L=the upper limit of population.

It is known that $b>0$ is the proper constant to a fiexed population and h is large under an unfavourable condition and the relation

$$L=b/h$$

holds.

For small x this is not much different from the exponential law but now as x tends to infinity the population size (if $b>0$) approaches to the upper limiting value L. Laws of growth of the form (2) have been used with success to describe the growth-phenomena displayed in many sets of circumstances, both by the lower organisms and by human populations.

It is known that equation (2) may be applied also to the growth phenomena of weight or length of individuals. In this case the meaning of the coefficients b and h are not necessarily the same as in the population growth and the coefficient b represents the intrinsic growth rate of biomass of individuals and h the effect of environment. Moreover it is known that for the growth of successive years the growth of individuals may be represented by the augumented logistic curve.

From data given in Table 1, using the mean weight of N individuals of fish in each pond, the differences between the initial weight $\underline{y}$ and final weight $\bar{y}$ of the first year were calculated*). For this case equation (2) takes on the form

$$y=\underline{y}+(\bar{y}-\underline{y})/[(1+exp(a-bx)] \qquad (3)$$

where b corresponds to the proper growth rate of the species in question and this type of equation is kown as the augumented logistic curve.

For the validity of the logistic curve Kostitsin[1]) gave an example which occures in the numerical results of the experiments of Gause on the culture of Paramecium and he concluded that this is more significant than the close agreement between the theoretical curve and the experimental curve.

The growth rate dy/dx was calculated from the data given in Table 1, from which it was found that the values of b are almost constant in each pond and they have not been influenced by the area of pond (A) and the number of individuals (N), accordingly b is independent of the density of population**). On the other hand the value of h, obtained from the relation $L=b/h$, is inversely proportional to the density and it seems that the area of pond slightly affects the value of h.

(iii) The effect of population-density on the fecundity of fish

At the spring of 1952, fish attained the age of three year old. The measurements of length and weight of fish were done on May 27, 1952. On a part of them the total and the body length, the height and the width of body and the weight of egg were measured. Now all the fish are matured and we may distinguish their sex. The results of measurements are shown in Table 1,

* Because the upper and the lower limits of body weight could not be obtained exactly in this experiment the first and the last observed values were substituted for these values. The discrepancies caused by these approximations will be small and will not cause any large errors in the following calculations.

** It was confirmed that b is independent of A and N by the analysis of variance. The method of calculation of the constant b is as follows.

Take the interval of observation as unit time Δx and corresponding increment of body weight $\Delta(y-\underline{y})$, then equation (1) becomes

$$\Delta(y-\underline{y})/\Delta x \fallingdotseq b(y-\underline{y})[1-(y-\underline{y})/L].$$

Next take y_1, y_2, y_3 and y_4 as the observed values of body weight on 0-th, 50-th, 100-th and 150-th days observations and put

$$u_i=(y_{i-1}+y_i)/2 \text{ and } u_i'=u_i/(\bar{y}-\underline{y}),$$

then from these relations we get

$$\Delta(y-y)/\Delta x \fallingdotseq b\ u(1-u')$$

from which values of b were calculated. Other methods of calculation gave similar results but are omitted.

Table 4. Measurements of weight, length of fish and number of egg.

A

No. of pond	Sex ♀	Sex ♂	Weight	Total length	Height	Width	Condition factor	0.85× TL.BH.BW.
			g.	mm.	mm.	mm.	F.	
III	♀		964	384	108	67	17	0.418
VI	〃		363	284	75	50	18	0.397
	〃		750	245	102	64	18	0.379
VII	〃		930	386	96	62	16	0.476
	〃		671	330	92	57	18	0.457
		♂	862	372	100	59	16	0.462
		〃	585	336	90	47	15	0.443
		〃	357	304	75	40	13	0.493
VIII		〃	506	329	80	53	14	0.426
		〃	378	304	73	46	13	0.436
		〃	468	315	77	46	15	0.494
		〃	383	286	80	50	16	0.394
		〃	356	303	70	47	13	0.433
		〃	262	260	70	40	17	0.425
		〃	337	286	70	41	14	0.483
		〃	255	254	68	42	17	0.413
	♀		630	330	100	64	17	0.350
	〃		469	289	86	55	19	0.403
	〃		469	322	80	45	14	0.475
	〃		337	266	72	46	19	0.450
	〃		300	265	67	40	18	0.496
	〃		352	290	75	46	14	0.414
	〃		562	330	82	52	16	0.470
	〃		431	312	75	47	14	0.461
	〃		413	300	74	45	16	0.487
	〃		401	290	76	44	16	0.486
IX	〃		321	270	70	45	18	0.443
	〃		381	258	80	47	25	0.431
	〃		246	262	72	46	15	0.338

B

No. of Pond	Sex ♀	Weight	Total length	Body length	Height	Width	Weight of egg	Number of egg	Weight of one egg
		g.	mm.	mm.	mm.	mm.	g.		mg.
III	♀	964	384	—	108	67	102	125×10^3	—
VI	♀	363	282	244	75	50	58.5	59.2 〃	0.990
IX	♀	321	270	230	70	45	41.6	56.6 〃	0.748
	♀	381	258	235	80	47	59.1	52.6 〃	1.125
	♀	246	262	220	72	46	48.5	59.4 〃	0.819

Table 4 and Table 5, from which we know that the sex ratio of fish in each pond is about 1 : 1. Although at the beginning of the experiment we could not know the sex of fish and we selected them randomly, the results of the measurements show that they were chosen suitably in respect to the sex ratio.

Table 5. Difference of weight and total length by sex.

No. of Pond	III	IV	V	VI	VII	VIII	IX	Total
				Mean weight of fish in gram				
Female (uncor.)	.1335	878	732	678	802	488	455	
(corrected)	1177	—	653	594	668	469	365	
Male	1072	—	554	567	605	369	344	
Diff. (D)	263	—	178	111	197	119	111	
Egg (E)	200	132	110	102	120	73	68	
(D′)	105	—	81	27	63	19	21	
(E′)	177	—	95	89	102	70	55	
				Total length of fish in cm.				
Female	416	366	347	347	358	299	299	
Male	391	—	331	332	337	292	228	
Diff.	25	—	16	15	21	7	21	
				Number of fish used				
Female	4	2	2+3	7+10	2	4+6	15+18	73
Male	8	0	5	14	3	8	30	64

E (E′): The estimated (corrected) weight of egg calculated from mean body weight.

In order to estimate the number of eggs of fish in each pond the body weight of fish were calculated according to their sex. From Table 5 it is evident that the weight of female fish is greater than male fish as the measurements were done on matured fish. On the other hand the weight of eggs and number of eggs were determined on five fish.*) From these figures (Table 4. B) the percentage of weight of eggs to body weight are obtained as 10.6, 16.1, 12.9, 15.5 and 19.7 hence the mean value is about 15 precent. Multiplying this value to the mean weight of female fish given in Table 5 the weight of eggs of these fish may be estimated and are given in the fourth row of Table 5. These figures are not equall to the diffence between the weight of female and male fish. Denoting above differences by the letter D we may obtain corrected value of D as follows:

* In order to continue the experiment few fish were used to the determination of the number of eggs.

$$D' = \left[\begin{array}{c}\text{Mean body weight}\\ \text{of femal fish}\end{array}\right] \times \left[\begin{array}{c}\text{Mean body length of male fish}\\ \text{Mean body length of female fish}\end{array}\right]^3 - \left[\begin{array}{c}\text{Mean body weight}\\ \text{of male fish}\end{array}\right]$$

Using modified value the corrected value of estimated weight of eggs per female fish is given by the formula

$$E' = 0.15 \times [\text{corrected weight of female fish}]$$

During winter season between 1951–1952 all the fish of Pond No. I and Pond No. II were died and in Pond No. IV only female fish were survived. Thus we can not compare the results for all ponds but arranging them according to the position of pond in the experimental field we get Table 6. In this table as the figures for Pond No. VII are too high, though its reasons arenot evident, the mean vslues were calculated excluding these values.

Table 6. Values o D, D′, E and E′.

Arrangement of pond in the exp. field			D			D′			E			E′		
Group			A	B	C	A	B	C	A	B	C	A	B	C
I	IV	VII	—	—	(197)	—	—	(63)	—	132	(120)	—	—	(102)
II	V	VIII	—	178	119	—	81	19	—	110	73	—	95	70
III	VI	IX	263	111	111	105	27	21	200	102	68	177	89	55
Mean			263	144	115	105	54	20	200	115	71	177	92	62

In Table 6 the population-density of pond in each colum are 0.51, 1.02 and 2.04 fish per square meter. If we plot the data on the logarithmic paper showing the relation among the population-density of fish in the pond of each group (i. e. A, B and C) and D, D′, E and E′, the points arrange on the straight line approximaltely. As the weight of one egg is about one mg. the number of eggs per female may be estimated from the weight of eggs. In Fig. 2 the relation between the logarithm of two variables may be shown by a straight line, as a first appromximation, e. g. by the form

$$\log Y = a + b \log X \qquad \text{or } y = AX^b \tag{4}$$

where X represent the density of fish in each group and Y represent one of D, D′, E or E′. Same results were obtained by Terao and Tanaka[5,6] (1928) and Kawaziri[4] (1949).

There are another ways to examine the effect of population density on the fecundity of fish. One of them is to determine the number of offsprings of

female fish. After the measurement on May 27, 1952, number of fish in each pond were changed in order to get better result on the hatching of eggs as shown in Table 7. Each pond contained two times of male fish to female fish as this ratio was recommended by expert to get best result. Excess of female fish were removed from each pond. Fish spawned on May 30 and

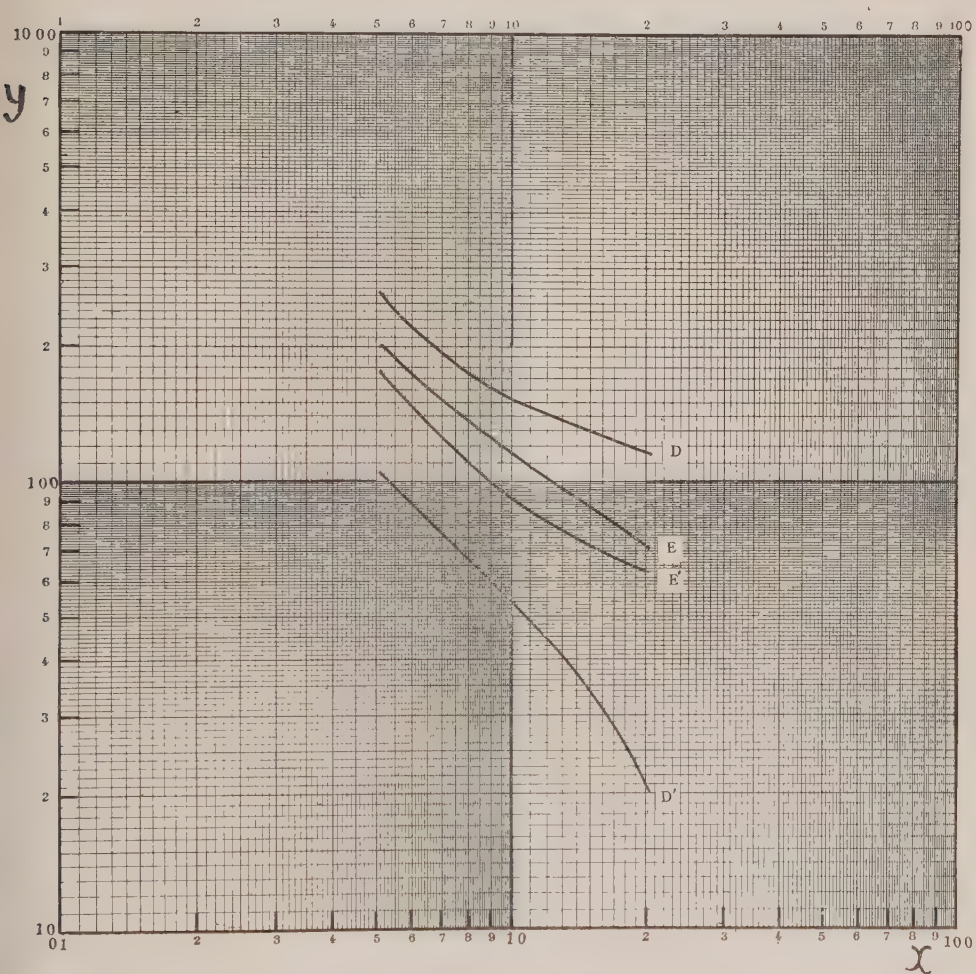


Fig. 2. Relation between the fecundity and density.

eggs hatched on June 3 and 4. Counting of larva was carried out on June 14. During this period a part of them were died consequently the figures on the last row in the table are survived number of larva. Eggs of Pond Nos. IV, V and VI were hatched but before the counting was done they were lost by heavy rain. Immediately after the spawning, samples were taken to get the percentage

Table 7. Percentage of dead eggs and number of hatched larva.

No. of fish	III	IV	V	VI	VII	VIII	IX
No. of fish	12	2	6	21	5	12	45
female	4	2	2	7	2	4	15
male	8	—	5	14	3	8	30
No. of dead eggs	—	232	60	40	144	—	111
No. of total egas sampled		261	333	333	733		581
No. of larva survided until Jun 14	—	—	—	—	1922	—	444

of dead eggs. From the results given above the definite relation betwesn these values of percentage and the density of fish population was not obtained. On the other hand the offsprings of fish *per capita* under lower density seems to be greater than the offsprings of fish under higher densty. The reason that the number of larva *per capita* is too small in this experiment must be caused by the insufficient maturity of a part of fish in each pond and the low hatching rate of eggs. As fish has about 50×10^3 eggs, more offsprings would be obtained if the hatching-rate is high. The auther intend to get better results in the experiment on next year than the results given here which are not satisfactory.

5. DISCUSSION

(i) In 1940, Feller[1] examined the recorded agreement between the logistic and actually observed phenomana of growth and concluded that a good agreement does not produce any significant evidence in favour of the logistic, beyond the plausubility of its deduction. He negated the conclusion of Kostitzin[1] which based on the experiment by Gause and stated that a safe evidence in favour of the logistic form is not even to be deduced from the experiments by Gause, and in no special case any biological conclusion must be based solely on an agreement between the observed data and the logistic form. Feller gave similar relations by means of randomly chosen growth curves and stated that the point is simply that the logistic form depends wholly on its mathematical advantages and the great plausubility of the assumptions leading to the differential equation. He also wrote that the crucial test for the theory of growth would consist in a systematic study of the parameters involved when external conditions are changed. The results obtained in this study will clarify the biological nature of individual growth because it is based on the whole processes of one growth cycle and some of the features which control it and conclusion does not depend upon the goodness of fit. Therefore objections such

as given by Feller to Gause's results, in the case of population growth, will be avoided.

(ii) In the study of the growth of whitefish of the Trout lake, Hile and Deason[3] concluded as follows;

Comparing with the growth of the whitefish of seven lakes, it was shown that the Trout lake whitefish has the lowest growth of any of seven populations. The great density of the population of the Trout lake appears to offer the most logical explantion of the slow growth of the whitefish. Wheather the crowding impedes growth through the creation of intense competition for food or through the operation of a "space-factor", whereby crowding hinders growth independently of competition for foods, remains to be detemined.

From the results of this experiment we may explain partially these questions. As stated above, the effect of density seems to have a dominant effect on the growth of fish. Hence we are inclined to attribute the slower growth rate of the Trout lake whitefish to the space-effect. However, since the phenomena are very complicated we may look for other possible explanations such as the effect of physical conditions of lake water or abundance of food etc.

(iii) In equation (3) we used $\underline{y}$ ann $\bar{y}$ as approximate values but it may be justified as follows:

Dividing both sides of equation (2) we can derive a transformed form of equation (2)

$$z=\frac{1}{1+\exp\ (a-bx)}\ , \qquad \text{where} \quad z=y/L.$$

The values of z varies from 0 to 1 as x ranges from $-\infty$ to $+\infty$. The first derivative of z is

$$\frac{dz}{dx}=\frac{b\ \exp\ (a-bx)}{[1+\exp\ (a-bx)]^2}\ .$$

Thus we may treat the variable x as if it defines a probability distribution and the derivative dz/dx a frequency function. The mean and variance of x are as follows:

$$E(x)=\int_{-\infty}^{\infty} x\ \frac{dz}{dx}\ dx=\frac{a}{b}$$

$$\text{Var.}\ (x)=\int_{-\infty}^{\infty}\left(x-\frac{a}{b}\right)^2\ \frac{dz}{dx}\ dx=\frac{\pi^2}{3b^2}$$

Therefore using the normalized variable

$$t=\frac{x-E(x)}{\sqrt{\text{Var. }(x)}}=\frac{\sqrt{3}}{\pi}\,b\left(x-\frac{a}{b}\right)$$

we get

$$\text{Pr. }(-\sqrt{3}<t<\sqrt{3})=\left|\frac{1}{1+\exp\left(-\pi t/\sqrt{3}\right)}\right|_{-\sqrt{3}}^{\sqrt{3}}$$

$$=\frac{1}{1+e^{-\pi}}-\frac{1}{1+e^{\pi}}$$

$$=0.9586-0.0414=0.9172$$

$$=1-0.0828$$

Thus we know that fish grow in summer about 90% of its total growth during a year if the period from May to October is $2\sqrt{3}$ times as long as the standard deviation of its growth curve. In this experiment the standard deviation of growth curve is

$$\pi/\sqrt{3}\,b\fallingdotseq\sqrt{3\pi}/2\times 3\fallingdotseq 0.91 \text{ (in unit of 50 days)}=46 \text{ days}$$

where the value of b is given in Table 1 and there is 151 days from May 18 to October 15. Consequently the ratio is $151/46\fallingdotseq 3.3$ which is very close to $2\sqrt{3}=3.46$.

In the above calculation an approximate value of b is used but it will not produce any significant error and we may fitly conclude that fish grow about 90% of its total growth in a year during the experimental period.

6. SUMMARY

I have cultured fish in nine ponds. The experiments undertaken in this study were designed to test the effect of density of population on the growth rate of fish. In the experiments, an attempt has been made to discover whether a fish is at a disadavantage in a pond of higher density and the results were discussed by means of the logistic law. Such a disadvantage might be indicated by its low growth rate and it was found so.

On the other hand Kostitzin had proved a validity of the logistic law when applied as a growth curve, for which Feller has criticized. His objection to Kostitzin's result is that a good fitting of the curve and consistency of the upper limit of the logistic curve do not show the validity of the curve, but it will not be adapted for the present experiments where the results depend on the relation of the parameters involved when external conditions are changed under assumption that in the logistic law the coefficient b and h have similar meanings as in the population growth when applied to the individual growth. Thus we may concluded that the present experiments provided an experimental verification for the validity of the logistic curve.

LITERATURES

1) KOSTITZIN, V. A. 1937. Biologie mathématique. Paris, A. Colin.

2) FELLER, W. 1940. On the logistic law of growth and its empirical verification in biology. Acta Biotheoretica 5(2), 51-66.

3) HILE, R. and H. J. DEASON. Growth of the whitefish, *Coregonus clupeoformis* (MITCHILL), in Trout Lake, North Eastern Highlands, Wisconsin. Trans. Amer. Fish Soc. 64(1934), 231-237.

4) KAWAJIRI, M. 1949. Influence of population density upon reproduction on Killiefish. Bull. Jap. Soc, Sci. Fish. 15(4), 167-172.

5) TERAO, A and T. TANAKA. 1928. Influence of population density upon the rate of reproduction in water-flea, *Moina Macropa* STRASS. J. Imp. Fisheries Inst. 24(1).

6) TERAO, A and T. TANAKA. 1928. Influence of population density upon the egg laying on the fish *Oryrias Latipes* T. & S. J. Imp. Fish. Inst. 24(1).

7) YOSHIHARA, T. 1952. A table and a alinement chart to facilitate the calculation of the condition factor Journ. Tokyo Univ. Fisheries. 39 (1), 63-67.

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

A TABLE AND A ALINEMENT CHART TO FACILITATE THE CALCULATION OF THE CONDITION FACTOR

Tomokichi Yoshihara

(Received on Sept. 30, 1952)

It is customary for fisheries investigations to express the fatness of fish body by the equation

$$F = 1000\ W/L^3 \qquad (1)$$

where W is the weight in gram and L the length in centimeter. This quantity is called the condition factor and may be considered as a number that describes the robustness of fish. The application of this cube law in computing a coefficient of condition of fish has been used by many workers. Recently Phenicie and Bishop[1] made alinement charts which represent the relationship among the body weight, the body length and the condition factor, but are constructed in yard-pond system though the values F, L and W are chosen to give the desired degree of accuracy. They stated that values from 10 to 100 would cover the needed range for F values and still give a F value of three significant figures.

In Japan, as scientific measurements are represented under the metric system, their charts can not be applied. Therefore a chart which is appropriate to the metric system was made. On referring to many works on the growth of fish it was found that in almost all cases the range of condition factor varies from 5 to 50 and the range of body length from 5 to 150 cm.

Assume that there is a cube which each side is 1 cm. and consists of a matter of specific gravity 1, then the condition factor of this cube is $1000\ W/L^3 = 1000$. Next suppose that there is a tube and radius of it is one-twentieth of its length, then the volume is $(L/20)^2 L\pi$, therefore the condition factor is $1000\pi/400 \fallingdotseq 10$.

According to Dr. Kimura[2] the condition factor may be defined by the equation (1), then we have the value $F = 1000$ for the cube and $F = 10$ for the tube. In practice, most of the values of F are enclosed in the range from 5 to 50 as stated above. The completed alinement chart is shown in the Chart 1 at the end of this paper.

Computation of F values from the the alinement chart is simple. A transparent straight-edge is placed on the length and weight values for any particular fish, and the F value is read directly from the condition factor scale.

The chart is very convenient to a rough and rapid calculation but the

exact F value can not be obtained. A table which enables us to compute the exact F value was made to get clear of the defects of chart. In Table 1 the values of $1/L^3$ are given. If we have a fish of W g. and L cm. we may calculate the F value from the table by only one multiplication. The table gives $1/L^3$ values of four significant figures and these figures will give sufficiently precise values for practical purpose. The order of figures must be determined suitably as shown in the following examples or by the chart.

Table 1. Table to facilitate the calculations of the value of condition factor. (This table gives the value of $1/L^3$. The figures are shown by significant figures.)

	0	10	20	30	40	50	60	70	80	90
0.0	—	1000	1250	3704	1563	8000	4630	2915	1953	1372
0.5	—	8636	1161	3525	1505	7764	4517	2854	1917	1349
1.0	1000	7513	1080	3357	1451	7536	4405	2794	1882	1327
1.5	2963	6575	1006	3199	1399	7321	4299	2736	1847	1305
2.0	1250	5787	9390	3052	1350	7112	4196	2680	1814	1284
2.5	6398	5120	8780	2913	1303	6911	4100	2624	1781	1263
3.0	3704	4552	8217	2872	1258	6716	4000	2571	1749	1243
3.5	2332	4065	7704	2660	1215	6532	3906	2518	1718	1223
4.0	1563	3644	7236	2545	1174	6349	3815	2468	1687	1204
4.5	1097	3280	6798	2435	1135	6177	3727	2418	1657	1185
5.0	8000	2963	6398	2332	1097	6010	3642	2370	1628	1166
5.5	6010	2685	6031	2235	1062	5848	3559	2323	1600	1148
6.0	4630	2441	5688	2143	1027	5695	3478	2278	1572	1130
6.5	3642	2226	5373	2056	9950	5543	3400	2234	1545	1113
7.0	2915	2035	5081	1974	9634	5400	3324	2191	1519	1096
7.5	2370	1866	4808	1896	9328	5260	3252	2148	1493	1079
8.0	1953	1715	4556	1822	9042	5126	3181	2107	1467	1062
8.5	1628	1579	4320	1752	8764	4995	3111	2067	1443	1046
9.0	1372	1458	4100	1686	8503	4869	3044	2028	1418	1031
9.5	1166	1349	3896	1623	8244	4748	2979	1990	1395	1015

EXAMPLES

Let the weight and length of fish be 300 g. and 25 cm. respectively. From Barlow's table we get $1/25^3 = 1/15625 = 0.00006400$. In Table 1. significant figure 6400 (after rounded) is shown and we may obtain $1000 \times 300 \times 0.00006400 = 19.2$. Thus the value of F is 19. A concise table which is merely represented at the interval of half centimeter is given for the sake of simplicity.

For fish whose length is smaller than 10 cm. the chart may be applied too. Multiplying the factor 10^3 on the denominator and numerator, equation (1) takes on the form

$$F = 1000\,W\,1000/(10L)^3 = 1000\,W'/(L')^3 \qquad (2)$$

where W′ in kg. and L′ in dm. As an example, assume that it is required to obtain the value of fish of which length is 8 cm. and body weight is 7.86 g. Suppose that weight is represented in kg. and length in dm. then applying equation (2) we get F=15 which is required value.

Table 2. The distribution of values of the condition factor.
(i)—(viii) show the date given below.

No. of pond	Date	Values of condition factor									
		0-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-	Mean
III	(i)				1	6	6	1	1	3	19.94
	(ii)				2	7	8	1			18.38
	(iii)				6	8	3	1			17.38
	(iv)				7	8	2	1			17.16
	(v)		2	10	5	1					14.08
	(vi)			3	8	7					15.94
	(vii)				3	9	5	1			17.94
	(viii)			2	7	5	4				16.82
V	(i)			1	3	2	5		1		18.00
	(ii)			1	7	3		1			16.35
	(iii)				1	5	4	1			19.00
	(iv)				2	6	1	3			18.13
	(v)		1	6	3	1	1				14.67
	(vi)			2	2	5	2				16.17
	(vii)			1	2	5	3	1			17.67
	(viii)			1	3	4	4				17.33
VI	(i)				11	18	7				17.16
	(ii)	1	1		10	18	5			1	17.16
	(iii)				1	11	12	12			20.42
	(iv)				5	9	13	14			20.50
	(v)		5	15	14	2					12.57
	(vi)		1	15	14	4	1				13.67
	(vii)			2	8	17	8				17.50
	(viii)			1	11	15	7				17.33
VIII	(i)					3	8	11	1	1	20.58
	(ii)				6	9	8				17.67
	(iii)				9	10	5				17.17
	(iv)		1	1	8	7	6	1			17.08
	(v)		2	13	5	4					14.42
	(vi)		1	3	10	7	2	1			16.25
	(vii)		1	5	8	9					15.92
	(viii)			3	6	12	2		1		16.92
IX	(i)			1	9	28	25	7	2		18.45
	(ii)			7	26	23	9	2		1	16.86
	(iii)	1	1		13	31	21	5			17.81
	(iv)	1			8	22	32	8			18.50
	(v)	2	8	32	26	4					14.11
	(vi)		1	14	34	18	5				15.93
	(vii)			3	13	32	13	6	3	1	18.03
	(viii)		1	7	21	30	17				16.92

Date: (i) May 18, (ii) July 7, (iii) Aug. 26, (iv) Oct. 15, 1950.
(v) June. 13, (vi) Aug. 1, (vii) Sept. 20, (ix) Nov. 9, 1951.
Ponds which are not shown in the table have small number of fish.

APPLICATION

In the previous report[3] the growth of carp was studied. From May 18, 1950 to Nov. 9, 1951 nine groups of carp were cultured in nine ponds under different area and density. Weight in each group of fish were obatined in fifty days intervals and mean body weight in each group of fish were calculated. From raw materials of measurement the condition factor of each fish in each period were calculated. Most of the values varies from 15 to 20. The distribution of

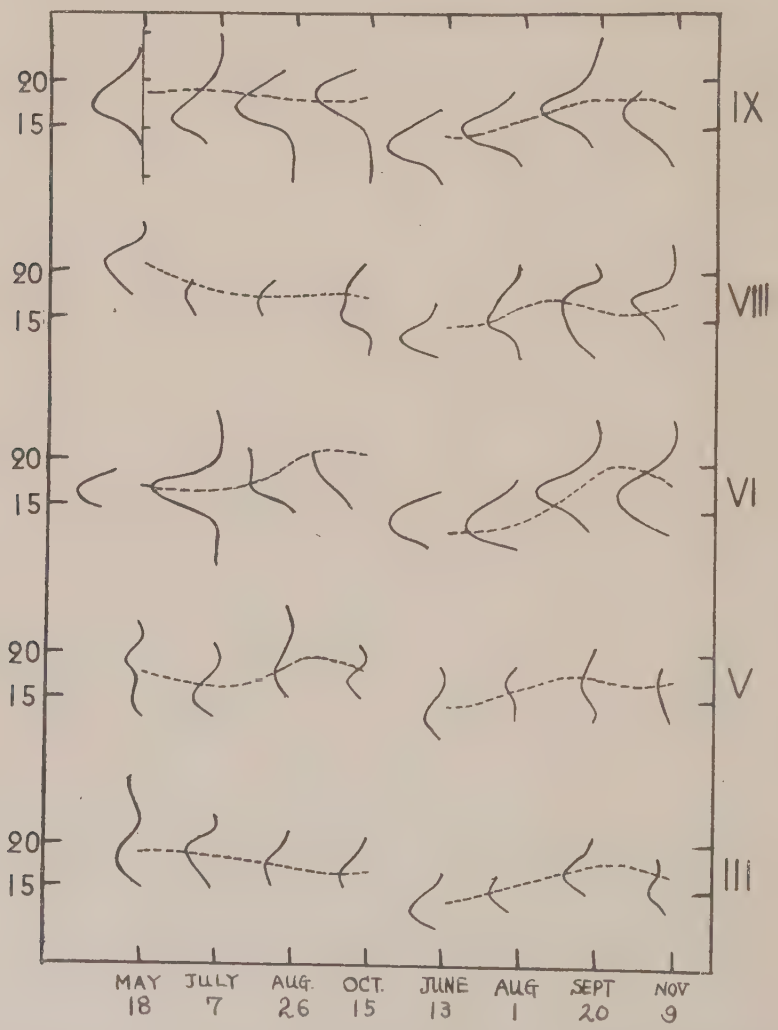


Fig. 1. Distribution of the values of condition factor at eight measurements. Dotted lines show the trend of mean value of F in each year. Figures in the left side are the values of F and No. of pond are shown in Roman type.

these values is shown in Table 2 and Fig. 1. On the other hand the weight of fish decreased in winter but the length increased. And this phenomena were confirmed by the examination of the values of the condition factor between the mean values of F on Oct. 15, 1951 and June 13, 1951 in each pond. There are considerable changes in the values of F within the year. At the same time under natural conditions similar phenomena are observed. It should be noted that in this experiment no body length of fish was measured except one case. Thus the values of F were obtained from the total length. Let L be the total length and L' the body length. From Table 4-B of previous report it was found that the average value of the ratio of L′ to L is about 0.85. Denoting F′ the values of the condition factor when the body length was used, we have the relation

$$F : F' = 1000\,W/L^3 : 1000\,W/(L')^3 = 1 : 0.85^{-3}\ 1 : 1.628$$

Therefore if it may be assumed the constant ratio of L : L′, we may use the results of Table 2 to compare the fluctuations of F values.

LITERATURES

1. Phenicie, C. K. and C. G. Bishop. Condition factor alinement chart. Prog. Fish-Culturist. 12 (3), 1950, 163-8
2. Kimura, K. Thickness and density of the fish body. Bull. Jap. Soc. Sci. Fish. 6 (2), 1937, 69-72.
3. Yoshihara, T. Effect of population-density and pond-area for the growth of fish. Jour. Tokyo Univ. Fisheries, 39 (1), 1952, 47-61.

Journal of the Tokyo University of Fisheries Vol. 39, No. 1, 1952

NOTES ON SOME MYXOSPORIDIAN PARASITES ON FISHES OF JAPAN

Toshikazu Hoshina

(Received Sept. 20, 1952)

In regard to the Myxosporidian parasitic to fishes in Japanese and adjacent waters, the investigations were made by several scholars, such as Fujita (1912–'36), Ishii (1915), Kudo (1915–'20), Ozaki & Isizaki (1941), Shiba (1934) and others and 11 general 79 species have been found up to the present.

The present paper gives descriptions of some species which appear to be new to science and additional informations on a few already known species of Myxosporidia. Before going further the writer wishes to acknowledge his indebtedness to all persons who were helpful for his work. His gratitude is first due to his respected teacher the late Nobutaka Nakai for his kind supervision. He is also deeply indebted to Dr. Yaichiro Okada and Dr. Terao Arata, who have kindly offered him facilities necessary for the study. His thanks are also due to Mr. Itirō Miyazaki for his kind supply of materials obtained by him in Kanazawa Bay, Kanagawa Pref.. Lastly the writer offers his hearty thanks to Dr. Satyû Yamaguti for the loan of literature from his private library.

MYXIDIIDAE

Myxidium enchelypterygii n. sp.

(Pl. 1, Figs. 1-3, Pl. III, Figs. 39, Tab. 1)

Habitat.—In the fins of the eel, *Anguilla japonica*. The myxosporidian parasite, *Myxidium sp.*, inhabting the fin of Japanese common eel, was first noted by Ishii[14] (1916).

Materials used for the present study were obtained from the Yoshida Fish-Cultural Laboratory in Shizuoka Pref., attached to the Tokyo University of Fisheries. Five infected fishes were captured in May, 1938 and two infected fishes in Sept., 1950. The infected fishes had a few white cysts (5~11) on the dorsal or anal or the both fins. The cysts lodged in the subepithelial connective tissue between the fin rays, usually in the basal part of the fin. The epidermis was slightly elevated by the cysts but any pathological changes of the host tissues were not found,

Vegetative Form.—The cysts are ellipsoidal in external view, measuring 0.52～1.35mm×0.38～0.83mm. Ectoplasm 4.3～5.0 μ thick. The peripheral zone of endoplasm is homogeneous, 3.3～6.8 μ thick and contains various stages of developing spores.

Towards the central portion, the structure is reticulated and filled with numerous matured spores. Pansporoblast mono-or disporoblastic. Polysporous.

Spore.—Broad fusiform in front and side views. The sutural ridge is not distinct, the sutural line straight and distinct. The shell is uniformly thick, with 6～8 longitudinal striations. Both sides of the shell are not equally curved. Namely one side is more swollen than another side.

The polar capsules are pyriform, equally large, lying at both opposite extermities. Coiled polar filaments indistinct under fresh or in the preserved state. Extruded polar filament 15～23 μ long. The extrusion of the polar filament is easily made under the treatment of the fresh spore with Lacto-Phenol Solution.

The sporoplasm located between the polar capsules is homogeneous and contains one or two nuclei. Dimensions of spore: length 7.13～9.00 μ, width 4.20～5.13 μ, thickness 4.00～5.10 μ, proportion of longth to width 1.43～1.82, polar capsules 1.73～2.40×1.50～2.10 μ. The details are compiled in the table 1.

Table 1. Dimensions of Spore.

Items	Numbers of spores measured	Range μ	Mean μ	S. D.
Length of spore	100	7.13～9.00	7.86±0.028	0.401±0.0198
Breadth of spore	〃	4.20～5.13	4.72±0.014	0.210±0.0100
Thickness of spore	〃	4.00～5.10	4.31±0.011	0.165±0.0018
Length: Breadth in spore	〃	1.43～1.82	1.70±0.008	0.112±0.0053
Length of polar cassule	200	1.73～2.40	2.11±0.006	0.132±0.0044
Breadth of polar capsule	〃	1.50～2.10	1.72±0.005	0.112±0.0038

Remarks.—The present species agrees with *Myxidium sp.* found by ISHII in the vegetative form, in the host species and in the seat of infection etc., but differs from that of ISHII's in the width of spore. ISHII's description is 7.7 μ by 2.8 μ in the dimension of the spore. Two other myxosporidian species parasitic to *Anguilla japonica*, belonging to the genus *Myxidium*, were recorded from Japan, viz. *M. anguillae* ISHII, 1915 and *M. matsuii* FUJITA, 1929. The present

species is clearly distinguished from those two species by the shape of spore and dimensions of the spore.

Myxidium matsuii Fujita, 1929

(Pl. I, Figs. 4-5, Pl. III, Figs. 40-42, Tab. 2)

This myxosporidian was first found by Fujita[8] (1929) in the integument of *Anguilla japonica* from the vicinity of Toyohashi, Aichi Pref.. About ten years ago, the writer had an opportunity to observe the skin disease of the eel due to the infection of a myxosporidian parasite probably referable to this species. In June, 1938, a explosive epidemic of the disease occured in an e l culture pond located at the outskrit of Odawara City, Kanagawa Pref. and a large number of eels, more than 100 kg in weight, were affected by the disease. Fifty specimens of the diseased fish were caught from the pond. They were 33～39cm long and 54～99gr. and showed numerous whitish cysts on the whole body surface except the head and each fins. However some of them had a few cysts on the head portion and on the basal part of the dorsal fin. Among the specimen some were found ulcerated and blooded on the skin. It seems that such pathological changes arose from the rupture of the riped cysts. The riped cysts were easy to break and discharge a milky white liquid, which contained numerous spores.

As the parasite gives rise to numerous cysts, the infected fishes show very tauching appearance. However, contrary to the external appearance, the influence of the parasite upon the vitality of the host fish is not sever. The infected flshes are not mortal and they may be recovered naturally, although, as above stated, the skin may be wounded by the rupture of the cysts, but when the contents of the cysts are discharged completly, the wound may be healed.

With the purpose of observing the course of the disease, ten specimens of the infected fishes were kept in the aquarium from June to July, 1938. The results showed that, the cysts began to fall out after the lapse of about 20 days and all the fishes were recovered after about a month. The water temperature during the experiment was 18～25° C, average 23.2° C.

Vegetative Form.—The cysts are located in the corium of the host's skin. They are circular or subcircuiar in an external view and slightly elevated on the skin, however, the cysts closely contacted with each other are irregular in outline. Dimensions of the cyst are 1.05～2.96mm by 0.66～2.54mm. In section preparations, they were circular or ovoidal or ellipsoidal. The ectoplasm

was 3.4～6.9 μ thick. On the inside of the ectoplasm, their was noted a thin layer which contained numerous nuclei and pansporoblasts under different stages of development and the central portion of it was filled with numerous spores. The fully developed cysts were filled with numerous spores and there was noticed the epithelium on the surface of the cysts disintegrated or fallen out. Besides these cysts, very young trophozoites were found rarely in the connective tissue of the host's skin. They were circular or ovoidal in outline, measuring 0.12～0.14×0.82～0.90mm and surrounded by numerous fibroblasts arranged concentrically. The cytoplasm was structurless and not clearly differentiated between ectoplasm and endoplasm. Along the periphery their was a comparatively wide zone of cytoplasm which had no nucleus, and on the inside of this zone the cytoplasm was dotted with numerous nuclei and a few sporonts. But the spores were not yet observed. As FUJITA already noted, there was observe the diffuse infiltration of spores in the subepithelial connective tissue of some parts of the host's skin, but it never occured in the muscular tissue. This indicates that the present species sometimes produces spores without forming a large trophozoite or the cyst.

Spores.—Broad fusiform in front and side views but in front view obtusely pointed at both ends. The shell is moderately thick and exhibits 14 to 16 longitudinal striations on the surface. The sutural ridge and the line are ndistinct. The polar capsules are equal in size and nearly spherical, the neck is obscure. The coiled polar filaments are also obscure in fresh and preserved materials.

Extruded polar filaments are 32～48 μ long, about four times long of the spore length. The sporoplasm is homogeneous, filling the remaining part of the intrasporal cavity, and contains two spherical nuclei, measuring 1.5 μ in diameter. Dimensions of spore are compiled in table 2.

Table 2. Dimensions of spore.

Items	Number of spore measured	Range μ	Mean μ	S. D.
Lenght of spore	100	9. 0～13. 8	11.75±0.043	0.632±0.0301
Breadth of spore	〃	6. 5～ 9. 0	7.70±0.051	0.610±0.0361
Thickness of spore	〃	6. 5～ 8. 5	7.27±0.027	0.402±0.0191
Length: Breadth in spore	〃	1.37～ 1.61	1.52±0.006	0.095±0.0046
Polar capsule in diameter	〃	3.60～ 5.52	4.35±0.035	0.521±0.0248

Pathological Changes.—The trophozoites of the present species invade the corium of the host fish and develop with forming the spores into the cysts. Seeing that the present parasite gives rise to large numbers of the trophozoite, the integument of the host fish is covered with the cysts, when they grow up. Further with the growth of the cysts the host's tissue may be pressed and the nutrition of the tissue may be hindered, so that it become necrotic and disintegrated. The most remarkable pathological change is the ulcreation accompanied with bleeding of the integument. It is caused by the disintegration of the crowded cysts. The figs. 41 (Pl. III) shows the state of the disintegration. The epidermis and the cysts are ruptured completely and there are found many spores and blood cells scattered in the disintegrated tissue. In the more advanced state, the epidermis and the cysts exfoliated and the fibrous connective tissue of the cutis become loosish, and pycnotic nuclei are seen in the tissue. The pathological changes are generally limited in the epidermis and the upper layer of the cutis. Sometimes, the lesions having the muscular tissue were observed (Pl. III, Figs. 40).

MYXOSOMATIDAE

Myxosoma (*Lentospora*) *dermatobia* (Ishii)

(Pl. I, Figs. 6-7, Pl. III, Figs. 43-44)

This myxosporidian species is the parasite of the integument of *Anguilla japonica*. A single specimen of *Anguilla japonica*, 59.0cm long, was taken from Lake Chuzenji, Nikko in Oct. 1932. The specimen preserved in formalin showed many lesions which were scattered on the whole body. These lesions resulted from the assembled cysts of the parasite (from a few to more than twenty cysts assembled in one lesion), measuring 2.3×2.6～14.7×7.2 milimeter, irregular in form and whitish in color. Some of them were ruptured and the connective tissues of the corium were exposed. Besides these concoursed cysts, a few isolated cysts were observed in places. They were subcircular or ellipsoidal in an external view, convexed and measuring 0.28～0.47mm×0.18～0.42mm. In section preparations, they showed an ellipsoidal form, surrounded by a thin membrane of the host origin and located in the corium.

The ectoplasm is thin and faintly striated; the peripherial endoplasm of the cysts is homogeneous and contains numerous nuclei, and sporonts, under various stages of spore formation, and towards the center, numerous matured spores are observable. Sporont mono-or disporoblastic, Polysporous.

The spore is subcircular in a front view; lenticular in a side or end view. Shell is smooth; 1.0~1.2 μ thick; in a front view 4 to 7 folds are seen along the sutural edge. Sutural ridge is prominent, comparatively broad. Two polar capsules are oval and convergent. Sporoplasm without iodinophile vacuole, contains one or two spherical nuclei measuring 1.5~1.7 μ in diameter. Dimensions of the spore as follows: length 7.12~8.10 μ, breadth 6.20~7.73 μ, thickness 4.30~5.12 μ, length of polar capsules 3.00~4.25 μ, breadth of polar capsules 1.94~2.33 μ.

Following two species referred to the genus *Lentospora* are found from the integument of *Anguilla japonica*. viz., *L. dermatobia* Ishii, 1916, *L. anguillae* Fujita, 1929,

Two genera *Lentospora* and *Myxosoma* were united and the genus *Lentospora* was referred to the genus *Myxosoma* by Kudo[19] (1933).

The present species agrees with the *Myxosoma* (*Lentospora*) *dermatobia* (Ishii) in every important characters and may be identified with this species although some discrepants are found in the dimensins of spore or in the parasitic status etc..

Myxosoma pseudorasborae n. sp.

(Pl. I, Figs. 8-10, Pl. III, Figs. 45 Tab. 3)

Habitat.—In the trunk muscle of *Pseudorasbora parva* (Temminck et Schelegel) from the lake Jônuma in Gunma Pref. The materials were collected by Mr. Morizumi Nakamura in June 12. 1937, and came under the writer's observation in a living state. Five infected fishes 4.0~5.4cm long showed one to three tumours on the dorsal or on the lateral or on the peduncle etc. The tumours measured 2.0~4.0mm×2.1~3.5mm and the microscopical observation revealed that each tumour was composed of a single cyst.

Vegetative Form.—The cysts were located in the muscular tissue of the host fish and surrounded by a connective tissue about 5 μ thick. They were spherical or ellipsoidal in outline, measuring 3.0~4.0mm×1.6~2.0mm. There were noted many sporonts under various stages of spore formation and young spores in the narrow pheripherial zone of the cyst, while an enormous number of spores occupied the greater central portion. Sporonts are monosporoblastic or disporoblastic. Polysporous.

Spore.—Subcircular or ovoidal in a front view; spindle form in a side and end view. The sutural ridge is comparatively broad; the sutural line distinct. The shell is smooth and uniformely thick.

A small intercapsular appendix present. Two polar capsules are ovoidal; equal in size and convergent. Coiled polar filaments have five to six whirls. When extruded they are 20~25 μ long. The sporoplasm occupies about one-half of the intrasporal space. It contains no iodinophile vacuole; the sporoplasm nuclei are spherical and two in number generally. Dimensions of the spore are compiled in the table 3.

Table 3. Dimensions of Spore.

Items	Numbers of spores measured	Range μ	Mean μ	S. D.
Length of spore	100	10. 8~14. 1	12.87 ±0.054	0.8050±0.391
Breadth of spore	〃	9. 9~12. 6	11.31 ±0.407	0.696 ±0.332
Thickness of spore	〃	5. 5~ 7. 9	6.48 ±0.355	0.516 ±0.246
Length: Breadth in spore	〃	1.04~ 1.35	1.129±0.0054	0.0805±0.00391
Length of polar capsule	〃	4. 0~ 6. 8	5.26 ±0.047	0.701 ±0.334
Breadth of polar capsule	〃	2. 2~ 3. 1	2.86 ±0.030	0.432 ±0.0210

Remarks.—The present form is distingushable from all known species of the genus *Myxosoma* in the dimensions of the spore. or in the shape of spore or in the host species and in the seat of infection etc. Therefore, the writer refers it to a new species provisisonally.

MYXOBOLDIAE

Myxobolus acanthogobii n. sp.

(Pl. I, Figs. 11-22, Pl. III, Figs. 46, Pl. IV, Figs. 47-49, Tab. 4)

This myxosporidian was first observed by Miyazaki* (1940) in the periphery of eye of *Acanthogobius flavimanus* (Temminck et Schelegel) form Kanazawa Bay Kanagawa Pref. But the writer observed the parasite not only in the part but in the cranium and in the vertebral column of the fish. Materials used for the present study were collected by Mr. Miyazaki in Sept., 1939 and those preserved in formalin, placed in March 1940 at the writer's disposal. They are six in all (body length 5.5~8.1cm) and besides the infection of this parasite, one fish was found parasitized by two species of myxosporidia in the gill of the fish, viz. one species of them is belonging to the genus *Henneguya* and the other seems to belong to a new genus. Two fishes out of the remaining five were infected also by the same species of genus *Henneguya*.

* Itirô Miyazaki.—1940: Jap. Soc. Sci. Fish., Vol. 9. p. 178.

The descriptions of these two myxosporidian species will be given in the latter section of the present paper.

Habitat.—In the orbit, cranium (all over the surface of brain, olfactory nerve etc.) and vertebral column (on the surface of spinal nerve) of *Acanthogobius flavimanus* from Kanazawa Bay. The infected fish showed the white coloured swelling of the periphery of both eyes (Pl. III, Fig. 46).

Aecording to MIYAZAKI (1940), the present parasites were found mostly in one year fish and the rate of infection showed about 80% in the fish measured 5.3~8.7cm in body length.

Trophozoite.—As above mentioned the parasites occur on the surface of cerebro-spinal nervous system, but they never infiltrate the tissues of brain or spinal chord. The trophozoites of the present species have not the form of ordinarly cysts such as the cysts of common histozoic myxosporidian species. They are polymorphic, dimensions are variable in large extent, measuring from 90.7~806.4 μ×75.6~504.0 μ, but the full-grown one shows the rounded form generally. The body of them consists of ectoplasm and endoplasm. The ectoplasm is thin and it's outer surface has numrous lamellated process. endoplasm of somewhat developed trophozoite is reticular and contains neumerous spores in the central part, and in the peripheral part, numerous nuclei and various stages of spore-formation are seen. The full-grown trophozoite is filled with spores, the pseudopodia degenerates and the ectoplasm is transformed into a membrane.

The endoplasma of young trophozoites is almost structureless and filled with numerous nuclei, sporonts, and sometimes a small number of spores.

The youngest trophozoite is a mononucleated body, polymorphic, measuring 5.85~11.90 μ×5.45~9.71 μ, and the ectoplasm has a few small pseudopodia, the endoplasm is granular and has spherical nucleus which is situated excentrigue. As the trophozoite grows up, the differentiation of ectoplasm and endoplasm becomes clear, and from the ectoplasm pseudopodia grow, and in the endoplasm numerous nuclei are formed and spores from these nuclei. The sporont is monoblastic or disporoblastic. The sporogony is similar to that of other myxosporidian species in the foudamentall respects. (see Pl. I, Figs. 17—22).

Spore.—Ovoid in a front view; lenticular in a side view and elliptical in an end view. The shell is smooth, 1.26~1.33 μ thick; sutural ridge and line are distinct, with 10~12 folds around the edge. The sutural plane coincides with the largest diameter of the spore. Two polar capsules are oval and convergent at the anterior end where occures a small intercapsular appendix.

The coiled polar filament is not distinct. The sporoplasm with a relatively large iodinophilous vacuole and two rounded nuclei measuring 1.00～1.20 μ in diameter. The dimensions of the spore is compiled in the table 4.

Table 4. Dimensions of the spore.

Items	Numbers of spores measured	Range μ	Mean μ	S. D.
Length of spore	100	7.98-11.84	9.848±0.0426	0.632±0.0301
Breadth of spore	〃	6.65～9.58	8.085±0.0361	0.555±0.0255
Thickness of spore	〃	5.32～7.18	6.162±0.0229	0.340±0.0162
Length: Breadth in spore	〃	1.06～1.40	1.217±0.0042	0.062±0.0029
Length of pole capsule	200	2.93～4.26	3.525±0.0105	0.221±0.0015
Breadth of pole capsule	〃	1.86～2.93	2.231±0.0086	0.181±0.0061
Diameter of iodinophilous vacuole	50	2.46～3.86	3.334±0.034	0.353±0.024

Pathological Changes—Although numerous trophozoites parasitize in the orbits, but the marked phathological changes are not proved in the various tissues of the eye. Only a few proliferative inflamations are observed in the tissues of seat of infection, viz. the proliferation of fibrous connective tissue and the infiltration of fibroblasts are seen in the lesions, so in the tissues of meninges, the congestion of blood is observed.

Although it is not clear how the host fish is under the influence of the parasite but judging from the seat of infection, it is conjectured that the parasite may be harmful for the host fish and the natural recover hopeless.

Remarks.—The myxosporidian parasite on cerebro-spinal nervous system of Japanese fish, one species, *Myxobolus* sp. was found by Dr. Yô K. Okada[22] (1932) from *Canthigaster rivulatus* collected at Misaki, Kanagawa Pref.. This species closely resembles the present form in the parasitic status or the shape of spore. But the Okada's description about the spore of the species is too brief, so the writer could not decide if the present form is identical or not with the Okada's form.

The spores of *M. pfeifferei*, *M. mülleri* and *M. squamosus* Kudo resemble those of the present species. But those species are distinguishable from the present form in the dimension of spores or vegetative form or host species etc. respectively. Hence the writer provisionally refered the present species to a new species.

Myxobolus koi KUDO, 1920

(Pl. I, Figs. 23, Textfigs. 1, Tab. 5-6)

This myxosporidian was first found by KUDO[18] (1920) in the gills of *Cyprinus carpio* from Tokyo Japan. Afterwards NAKAI[21] (1926) found a myxosporidian which may be referable to this species, in the same host species from the same locality and he dealed with the parasite as a new species and gave a detailed description on the parasite under the same specific name.

In Sept., 1943, a serious epidemic due to the parasite occured among the young carps (about four months old, body length 2.75~5.01cm, body weight 0.44~2.73 gr.) in the pond of the Yoshida Fish-Culture Laboratory in Shizuoka Pref., attached to the Tokyo University of Fisheries, and about fifty thousands of carps were found dead from the early of Sept. to the end of Oct. of that year. Some biological observations about the parasite, recorded in that occasion, are, as follows:

Habitat, trophozoite and the influence of the parasite upon the host fish.—The present species produces a conspicuous tumour in the connective tissue of the gill filaments of *Cyprinus carpio*. Sometimes the parasite is found also in the gills of *Carassius auratus*.

The tumours are massive, measuring more than 5mm in the largest diameter, and mostly they showed 2.4~3.7mm in the materials examined by the writer. They present a red color in a living state since the surrounding tissues are congestive. Usually they are seen externally, because the gill cavers of the host fishes are elevated by them.

In one gill lamella one to three cysts were found generally and in one fish, several cysts were found.

The table 5 shows the total numbers of the cysts found in 88 infected fishes collected at the Yoshida Fish-Cultural Laboratory in Sept., 1943.

Table 5. The total numbers of the cyst (tumour) found in the gills of 88 infected fishes.

Right Branchiae				Left Branchiae			
1st	2nd	3rd	4th	1st	2nd	3rd	4th
57	63	78	77	44	86	98	68

The tumours are composed of many grown trophozoites as NAKAI[21] (1926) stated already, therefore the section preparations show complicated structures

resulted from the proliferated connective tissues of host's origin around each trophozoite. The trophozites are polymorphic, and they vary to a considerable large extent in size, measuring 0.10～1.00mm in the largest diameter. These trophozoites are enveloped by a layer of connective tissue accompanying blood capilaries and they form a conspicuous tumour.

The ectoplasm is 3.5～6.2 μ thick, in the peripheral zone of the endoplasm are found many sporonts under various stages of development, towards the central portion, the endoplasm is filled with numerous spores. The sporonts are mono- or disporoblastic. Polysporous.

Although the parasite properly inhabits the gills of carp, and the spores are found at the same times in the various organs of the infected fishes. Eighty eight fishes of the diseased fishes were examined and following results were obtained (Table 6).

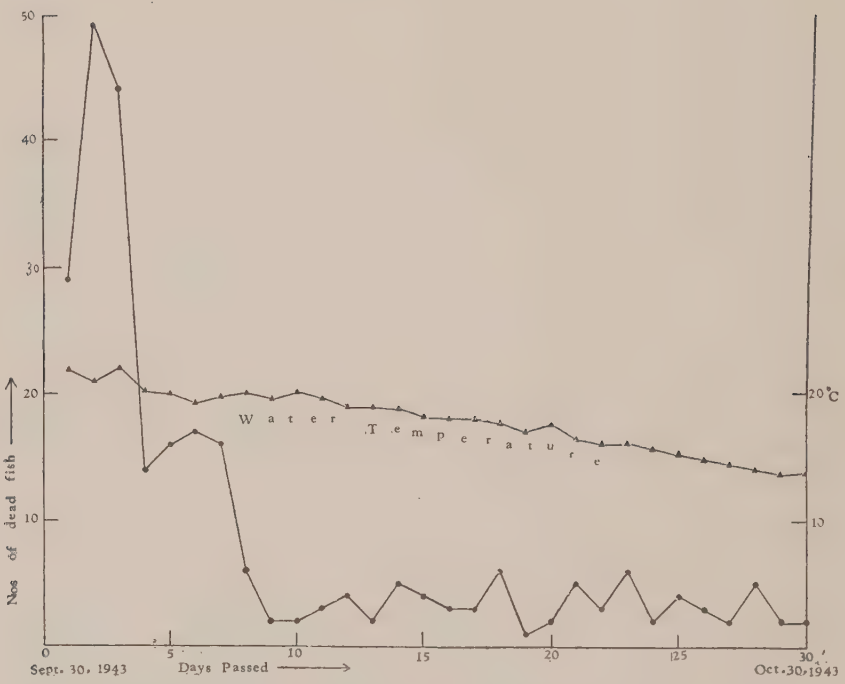
Table 6. Showing the numbers of fishes found to be parasitized by the parasite in their heart, kindey, liver and intestine.

Nos. of fishes examined	Nos. of fishes in which the spores were found in			
	Heart	Kidney	Liver	Intestine
88	41	48	35	44

The infulence of the parasite upon the host fish may be very severe, because the parasite gives rise to conspicuous tumours, so the surface of the gills become covered by them, or the tissues destroyed or the movement of the gill cavers are hindered, in the consequence the host fishes may be suffocated.

The diseased fishes crowd generally on the water surface, they become weak gradually and finally may be passed away without any noteceable symptoms. Nakai (1926) has been observed that the diseased fishes (2 year old) which were kept in a aquarium, recovered naturally except only one fish out of the 15 fishes. But in the case of young fish, the natural recovery is not so hopeful. The course of the disease was observed from Oct. 1, 1943 to the end of the month on the 300 diseased fishes which were kept in a small pond. During the period, 256 fishes were found dead. Remaining fishes lived over the end of Oct. and some of them showed the recovery from the disease in the course of the experiment.

While, in the case of large fish (more than two year old), the present parasite produces small whitish cysts in the gill filament, measuring less than 1mm and is not harmful for the host fish.



Textfigs. I. Daily mortality curve observed from Oct. 1. 1943 to Oct. 30, 1943. About 300 diseased fishes were kept in the small pond.

Spore.—The characteristics of the spores well agree with that of NAKAI's description. Dimensions of spores: length 10.3~13.4 μ, breadth 6.0~7.6 μ, thickness 5.8~6.8 μ, polar capsules 5.4~7.2 μ by 2.4~2.9 μ, polar filaments 48~62 μ long.

Some experiments on the control of the disease.—Whether the treatments of the diseased fishes with some chemicals are effective for control of the disease or not, some experiments were carried out by using following chemicals:

Chemicals	Concentration	Times of treatment
Sodium choiride	0.5%	60 mimutes
,,	0.25%	constantly
Cupper sulphate	0.05%	30 minutes
,,	0.025%	,,
Potassium permanganate	0.005%	,,
Glacial acetic acid	0.05%	,,
Phenol	0.0025%	,,
Methyle blue	0.005%	,,

Eosin	0.01%	,,
Formalin	1:5000	,,
,,	1:10000	,,

This experiments were carried out from Sept. 1 to Oct. 19, 1943 under the water temperature 26°~18° C.

In each experiment, ten fishes were used and treated once a day and the treatments were continued every day. But all the fishes died within ten days and there were not found any remedial effects in the treatments. Namely, the chemotherapies by such chemicals as mentioned above are not effective for control at certain advanced stages of the disease. But in the case of the treatment under low temperature (18~20° C), the phenol treated fishes showed the most prolonged living compared with the control fishes and the fishes treated with other chemicals (The control fishes lived three to five days, while the phenol treated fishes lived seven to ten days from the begining of the treatment).

Henneguya miyazakii n. sp.

(Pl. I, Figs. 24-25, Pl. IV, Figs. 50-51, Tab. 7)

Habitat.—In the integument of the gobioid fish, *Chaenogobius annularis annularis* (Gill), collected by Mr. Miyazaki in Sept., 1939 from Kanazawa Bay, Kanagawa Pref. and two infected fishes preserved in formalin were presented to the writer for investigation. One specimen, 4.6cm long, had 24 semispherical, whitish cysts on the dorsal, lateral and ventral side of the body (Pl. IV, Figs. 50) and the other one, 4.2cm long, showed 3 ruptured cysts, yet still contaning spores and many traces of natural recovery from the infection on the various locality of the trunk.

Vegetative Form.—The cysts were located in the connective tissue of corium. They were semispherical in general form, but some cysts developed in contact with each others were irregular and varied in size 1.06~3.80mm × 1.01~2.20mm. Several cysts were examined in section preparation, all of them were at advanced stages of development and filled with mature spores, yet in them there was noted a thin peripheral zone of endoplasm containing numerous nuclei and pansporoblasts at various stages of development (Pl. IV. Figs. 51). Sporonts are mono- or disporoblastic. Polysporous.

Some trophozoites were found occasionally to parasitize between the corium and the trunk muscle but these did not present a large form but showed a flattened and elongated form along the long axis of the host body. The

host epithelial tissue on the cyst was very thin, and there were no other remarkable pathological changes in the host tissue. When the parasite fully matured, the cysts break out and the contents are extruded and the natural recovery may be made, therefore the influence of the parasite upon the host may be not severe.

Spore.—Subcircular to ovoid with somewhat broad anterior half in a front view; broad fusiform in a side view. In a front view, there are noted two long tails forming an obtuse angle at the posterior end of the spore, and a fold runs from the base of tail to one third of the spore length in the middle field of the shell and six folds around the sutural edge.

The shell about 0.9 μ thick, consists of two equal valves; the sutural plane coincides with the largest diameter of the spore. Sutural ridge distinct, about 4.0 μ broad.

Two polar capsules are pyriform, equal in size and the anterior extremites cross each other. Coiled polar filaments are distinct in the preserved specimen, coiled 4~5 times. During the present study one spore which extruded a polar filament was found by chance, although the materials are preserved in formalin. The filament measured 18.6 μ long. The sporoplasm occupies about the two third of the intrasporal cavity and contains an iodinophile vacuole and two compact spherical nuclei, measuring 1.60~1.70 μ in diameter.

The dimensions of spores are compiled in the table 7.

Table 7. Dimensions of spores.

Items	Numbers of spores measured	Range μ	Mean μ	S. D.
Length of spore	100	9.64–13.30	12.00±0.051	0.761±0.0360
Breadth of spore	100	8.51~11.17	9.77±0.045	0.665±0.0317
Thickness of spore	55	4.12– 7.32	6.22±0.063	0.693±0.0446
Length of tail	100	33. 1~56.1	44.62±0.331	4.938±0.2356
Length: Breadth in spore	100	1.07~ 1.42	1.24±0.006	0.082±0.0391
Lenth of polar capsule	200	3.13– 4.26	3.72±0.016	0.326±0.0109
Breadth of polar capsule	200	2.00– 2.99	2.54±0.011	0.230±0.0078
Diameter of iodinophile vacuole	55	2.66– 4.26	3.46±0.016	0.337±0.0114

Remarks.—The shape of spore proper to the present species indicates affinities to that of *H. zschokkei*, *H. salmonicola*, *H. musslini*, *H. brachyura* and

H. miyairii, but the present species is sharply distinguished from all these in the characteristics of tail, namely the tails of the present species are projected to obliqueposterior directions, forming a large obtuse angle and although they are slender, stretched them-seleves tightly. Moreover the present form has a fold on the frontal surface of the shell at the middle posterior end of the spore.

Hence, the present form deserves to be considered as a new species. The specific name is given in honor of Mr. I. Miyazaki who collected the parasite,

Henneguya tridentigeri Ozaki & Isizaki, 1941.

(Pl. I, Figs. 26-27, Pl. IV. Figs. 52-53, Tab. 8-9)

Habitat.—The gills of *Acanthogobius flavimanus* (Temminck et Schlegel) from Kanazawa Bay. This parasite was found from the fishes which were parasitized by the *Myxobolus acanthogobii* n. sp. (see. p. 75). The infected fishes had white small cysts on the interrespiratory filaments of the gills. The cysts lodged in the subepithelial connective tissues of the gill filament. They counted 10~15 cysts per one gill lamella.

Vegetative Form.—Cysts were nearly spherical or ellipsoidal in external view, measuring 126.0~423.4 μ × 79.4~252.0 μ and were filled with developing and mature spores. The sporont is mono-or disporoblastic. Polysporous.

Spore.—Oval form in a front view, with a somewhat pointed anterior end and gradually attenuated towards the posterior extermity; broadest at about the level of anterior third of the spore length; spindle form in a side view. The total length of the spore is 43.8~62.6 μ, the spore proper, from the anterior end to the posterior margin of its cavity, is 10.4~13.7 μ, 5.9~8.6 μ broad, 4.2~6.0 μ thick and the proportion of the length to the breadth 1.44~1.80. The shell is uniformly thick, measuring 0.86~0.93 μ. The sutural ridge is narrow, with a indistinct sutural line.

Two posterior appendages are slender and long, separated from the near basal part, measuring 30.0~55.7 μ, 2.5~3.5 into length of the spore proper. Two polar capsules are pyriform, equal in size, and somewhat convergent. Coiled polar filaments are distinct in the preserved state, being six to seven coiled. Dimensions of polar capsules are 3.59~4.46 μ long, and 2.26~3.20 μ broad.

Dimensions of the spore are compiled in the table 8.

Remarks.—As indicated in table 9. the present species closely resembles

H. tridentigeri OZAKI and ISIZAKI, 1941, parasitic to the integument of *Tridentiger obscurus* (TEMMINCK et SCHLEGEL) from the river Ota in Hirosima district.

Table 8. Dimensions of spore.

Items	Number of spores measured	Range μ	Mean μ	S. D.
Length of spore proper	100	10.37~13.79	11.51±0.049	0.730±0.0349
Breadth of spore	〃	5.99~ 8.60	6.61±0.029	0.432±0.0207
Thickness of spore	〃	4.16~ 5.99	5.24±0.015	0.223±0.0106
Lenght: Breadth in spore	〃	1.44~ 1.80	1.53±0.007	0.101±0.0048
Length of tail	55	30.0 ~48.7	36.62±0.379	4.164±0. 268
Length of polar capsule	200	3.95~ 4.96	4.32±0.009	0.201±0.0067
Breadth of polar capsule	〃	2.26~ 3.20	2.65±00.06	0.182±0.0042
Diameter of iodinophile vacuole	60	2.00~ 3.19	2.59±0.028	0.316±0.0294

Table 9. Comparison of characteristics between the present species and *H. tridentigeri* OZAKI & ISHIZAKI.

Species / Items	*H. tridentigeri*	Present species
Host and Seat of Infection	*Tridendiger obscura*, in the Integument	*Acanthogobius flavimanus*, in the gill
Vegetative Form	Cyst, round or ovoid, 1.28 ×1.0 mm in largest size	Cyst, round or ovoid, 0.42 ×0.25 mm in largest size
Spore	Oval; the tail long and filamentaus	〃
Deminsions of spore:		
Length	12.5 μ	11.5 μ
Breadth	7.4 〃	6.6 〃
Thickness	7.3 〃	5.2 〃
Tail	41.4 〃	36.6 〃
Length of polar capsule	5.8 〃	4.3 〃
Breadth of polar capsule	2.2 〃	2.7 〃
Iodinophile vacuole	2.9×2.5 〃	2.6 〃

There are no remarkable differences between the two species with the exception of the differences in the size of vegetative form and in the seat of infection or in the host species etc. These differences, however, are too slight to distinguish the present species from *H. tridentigeri*, so the writer identified this species with OZAKI and ISIZAKI's species.

Trigonosporous acanthogobii n. g. et n. sp.

(Pl. II, Figs. 28-38, Pl. IV, Figs. 52-54, Tab. 10)

Habitat.—Gills of *Acanthogobius flavimanus* (T. et S.) from Kanazawa Bay, Kanagawa Pref. A single fish out of the already mentioned materials (p. 75) was found parasitized by this myxosporidian. The gills showed mixed infection of the present myxosporidian and *Henneguya tridentigeri* and 2 to 5 whitish cysts of the present species were found in the each gill lamella of the both branchiae. The cysts were surrounded by the epithelial tissue and the thin membarne (ca. 2.4 μ) of host origin and lodged in the subepithelial tissue of the gill filament.

Vegetative Form.—An exterior view shows that the cysts are subspherical or ellipsoidal, measuring 0.56~0.76mm×0.41~0.65mm. The cross-section is circular. The ectoplasm about 9 μ thick and fienly striated. The peripheral endoplasm is homogeneous and contains numerous nuclei, sporonts and pansporoblasts at various stages of development, while towards the central portion the endoplasm is occupied by numerous spores. The pansporoblast is disporoblastic. Polysporous.

Spore.—Viewed from front, the spores are nearly isosceles triangular, spindel form in posterior view and firly flattened. The anterior end is rounded; the base straight; the posterior ends of both sides of the two shell valves extended and formed the two filaments like appedages respectively, measuring 50~99 μ long, and these two pairs of appendages at each sides are connected respectively by a transversal filament (13~19 μ long), which is running parallel with the base of the spore at about three fourth of the entire length of the appendages from the posterior ends. The shell is smooth and about 1.0 μ thick; the two shell valves are symmetrical; the sutural ridge and line not distinct; the sutural plane coincides with the largest diameter of the spore. Two polar capsules situated at the anterior end of the spore are pyriform, equal in size and convergent. Coiled polar filaments are distinct in the preserved state, they are four times coiled.

The sporoplasm occupies the remaining greater part of the sporal cavity and contains an iodinophile vacuole and two spherical nuclei, measuring 1.60~1.72 μ in diameter. Two capsulogenous nuclei are also observable in the spore. Dimensions of the spore are compiled in the table 10.

Sporogony.—Although the details of the nuclear division and the other respectes were not determined as the materials were not fit for the cytological

Table 10. Dimensions of spore.

Items	Numbers of spores measured	Range μ	Mean μ	S. D.
Length of spore	100	7.31~10.50	8.74±0.0413	0.612±0.0292
Breadth of spore	〃	11.05~18.75	13.94±0.0624	0.925±0.0449
Thickness of spore	〃	4.66~ 6.12	5.50±0.024	0.352±0.0168
Length: Breadth in spore	〃	1.20~ 2.00	1.61±0.083	0.123±0.0588
Length of appendage	53	50. 4~99. 1	68.28±1.047	11.302±0.7418
Length of transversal appendage	〃	13.00~19.29	15.38±0.168	1.813±0.188
Lergth of polar capsule	200	3.06~ 4.50	3.81±0.013	0.264±0.0089
Breadth of polar capsule	200	2.00~ 2.93	2.49±0.009	0.182±0.0061
Diameter of iodinophile vacuole	60	2.66~ 4.79	3.33±0.045	0.524±0.0321

study, the outline of sporogony may be as follows: Along the peripherial zone of the cyst, the cytoplasm is dotted with numerous nuclei, they are spherical, measuring 2.93~4.66 μ in diameter, and with a large caryosom. The sporont may arise from these nuclei. By the first heteropolar division, the developing nucleus as a sporont divides into a small and a large nucleus. The two nuclear stage is followed by suecessively nuclear division and a definite number of the nuclei are produced in the sporont. The number of the nuclei showed thirteen. When the nuclei of the sporot have increased to a difinite number, the cytoplasm of the spornt begins to grow and before long the sporont transforms into a pansoporoblast. The young pansporoblast presents an oval form and as its's development advances the differentiation of the cells is proved viz. 4 capsulogenous cells, 2 sporoplasm (each of them contains 2 nuclei), 4 nuclei for shell valves formation and one nucleus remains as pansporoblast nucleus. These differentiated cells divide into two groups and cach two capsulogenous cells sits converging at both ends of the pansporoblast and below the capsulogenous cells occupy the sporoplasms respectively. With the advance of the sporulation, the pansporoblast dfferentiates into two sporoblasts and each sporoblast begins to form the shell valves and becomes to present a nearly triangular form. Posterior ends of each side of the shell valves extended outwards and the appendages of the spore are formed. By the time the formation of shell valves has been advanced as well as the polar capsules and also an iodinophile vacuole appears in the sporoplasm. Thus two spores are formed from the pansporoblast (Pl. II, Figs. 30—38).

Pathological Changes.—The respiratory filaments are pressed hard and atrophied by the developement of the cysts. The epithelial tissue of the gill filament also showed a great flattening under the influence of the cysts. The condition shows that the parsite may be not a little injurious to the host fish.

Remarks.—The present species should be classified under the fam. Myxobolidae because the sutural plane of the spore coincides with the largest diameter of the spore and the sporoplasm with an iodinophile vacuole.

The spore of the present species presents a nearly isoscels triangle and the posterior end of each side extended and formed two long filaments like appendages.

These appendages of each side joins to each other by a filament which is running parallel with the base of the spore respectively. And the present species, as compared with the known members of the family, resembles genus Henneguya in the shell valves having long appendages, but difiers fundamentally from the genus in the position, in the number and the structure of the appendages etc. Therefore the present form is easily distinguished from the genus and at the same time from all other known genera. For the reason the writer proposes here to establish a new genus *Trigonosporous* and to refer the present species as a type species of the genus to *Trigonosporus acanthogobii* n. g. et n. sp.

Trigonosporus n. g.

Generic Diagnosis.—Myxobolidae: Spore is nearly triangular in a front view; spindle in an end view; anterior end rounded; posterior end straight. Two long slender appendages are projected from the posterior end of the each side of the spore and each pair of the appendages joins respectively each other by a filament paralleled with ihe base of the spore. Two polar capules are situated at the anterior end of the spore. Trophozoit is polysporous and forms a cyst. Histozoic parasite of brackish fish. Type species: *Trigonosporous acanthogobii.*

SUMMARY

1. Five new species of Myxosporidia are described and an additional information on four known species of Myxosporida is given. They are: (1) *Myxidium enchelypterygii* n. sp. from the fins of *Anguilla japonica* Temminck et Schelegel, (2) *Myxidum matsuii* Fujita, 1929, (3) *Myxosoma* (*Lentospora*) *dermatobia* (Ishii), (4) *Myxosoma pseudorasborae* n. sp. from the trunk muscle of

Pseudorasbora parva (TEMMINCK et SCHELEGEL), (5) *Myxobolus acanthogobii* n. sp. from the orbits and cerəbro-spinal ne.vous system of *Acanthogobius flavimanus* (TEMMINCK et SCHELEGEL), (6) *Myxobolus koi* KUDO, 1920, (7) *Henneguya miyazakii* n. sp. from the integument of *Chaenogobius annularis annularis* (GILL), (8) *Henneguya tridentigeri* OZAKI & ISIZAKI, 1941 from the gills of *Acanthogobius flavimanus* (TMMINCK et SCHELEGEL), (9) *Trigonosporous acanthogobii* n. g. et n. sp. from the gills of *Acanthogobius flavimanus* (TEMMINCK et SCHELEGEL).

2. Brief informations on the skin-disease of the eel due to the infection of *Myxidium matsuii* FUJITA, 1929 and on the carp-disease due to the infection of *Myxobolus koi* KUDO, 1920 are noted.

3. An attempt to control the carp-disease by some chemicals is made but the treatments were not effective.

PAPERS CITED

1. FUJITA, T. 1912: Notes on New Sporozoan Parasites of Fishes. Zool. Anz. Bd. 39, pp. 259-262.
2. ———. 1913: On a New Species of *Chloromyxum* from the Gall Bladder of the carp Annt. Zoəl. Japan. Vol. 8, pp. 257-259. 1 textfig.
3. ———. 1915: Studies on Myxosporidian Parasites of Flat Fishes from Hokkaido (in Japanese). Zool. Mag. (Japan), Vol. 27, pp. 487-491.
4. ——— 1923: Studies on Myxosporidia of Japan. Jour. Coll. Agr. Hokkaido Imp. Uni., Vol. 10, pp. 192-248. 2 pl.
5. ———. 1924: Studies on Myxosporidian Infection of the Crusian Carp. Jap. Jour. Zool. Vol. 1, pp. 45-57. 1 pl.
6. ———. 1952: On two new Species of *Chloromyxum* found in the fresh-water Fishes in Sapporo. Ann. Zool. Japon. Vol. 10, pp. 277-280. 2 textfigs.
7. ———. 1927: Studies on Myxosporidia of Japan. Jour. Coll. Agr. Hokkaido Imp. Uni., Vol. 16, pp. 229-247. 1 pl.
8. ———. 1929: The skin-disease of the ell. Annot. Zool. Japan. Vol. 12, pp. 245-250. 1 pl.
9. ———. 1930: On a new Myxosporidian, *Chloromyxum bora* nov. sp., found parasitic in muscle of the graymullet of Formosa. Zool. Mag. (Japan) Vol. 42, pp. 45-48, 3 textfigs.
10. ———. 1936: Notes on some Myxoporidian in Fresh-Water Fishes (in Japanese). Zool Mag (Japan), Vol. 48, pp. 595-602. 1 pl.
11. Hoshina, T. 1949: On the new Myxosporidian parasite of the genus *Myxosoma*, *M. salmonis* n. sp., infecting on the scale of the dog salmon (in Japanese with English summary). Seibutu Vol. 4. no. 3, 106-109. 5 textfigs.
12. ISHII, S. 1915: Myxosporidiosis of Japanese eel (in Japanese). Zool. Mag. (Japan). Vol. 27, pp. 372-382. 1 pl.
13. ———. 1915a: Lentospora-disease of the eel (in Japanese). Ibid. Vol. 27, pp. 471-474. 4 figs.

14. ———. 1916: On a Myxosporidian parasite found parasitic in the fin of Japanese eel (in Japanese). Ibid. Vol. 28, pp. 271-273.

15. KUDO, R. 1915: On a new Myxosporidian from a carp (in Japanese). Zool. Mag. (Japan). Vol. 27, 517-523. 2 pl.

16. ———. 1916: Contributions to the study of parasitic protozoa. III Notes on some Myxosporidia found in some fresh-water fishes of Japan, with the description of three new species. Jour. Parasit. Vol. 3, 3-9. 4 figs.

17. ———. 1917: Contributions to the stndy of parasitic portozoa. II. *Myxobolus toyamai* nov. spec., a new Myxosporidian parasite in *Cyprinus carpio* L. Jour. Parasit., Vol. 3, 163-170; 2 pl.

18. ———. 1920: Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. III. Biol. Mnogr. Vol. 5, nos. 3 and 4, 1-265, 1 textfig. and 25 pls.

19. . 1933: A taxonomic consideration of Myxosporidia. Trans. Amer. Micro. Soc. Vol. 52, Nos. 3. pp. 195-216.

20. ———. 1934: Studies on Some Protozoan Parasites of Fishes of Illinois. III. Biol. Monogr. Vol. 13, No. 1, pp. 7-44. 8 pl.

21. NAKAI, N. 1926: Eine neue Myxosporidienart aus den Kiemen der Karpfens. Jour. Imp. Fish. Inst. Vol. 27, no. 1 pp. 7-16. 2 pl.

22. OKADA, YÔ K. 1932: Myxosporidie du Cerveaux et de la Moelle d'un *Canthigaster* (Poisson tiliostéen). 4 text figs. Bull. Soc. Zool. France. T. 57, pp, 39-44 4 textfigs.

23. OZAKI, Y & ISIZAKI, H. 1941: Myxosporidian Parasites of *Tridentiger obscurus* (TEMMINCK et SCHLEGEL). Jour. Sci. Hirosima Uni. Ser: B. Div. 1. Vol. 9. 181-192. 12 textfigs. and 4 pl.

24. SHIBA, N. 1934: The Morphology and Development of *Henneguya macropodi* n. sp. from a paradise-fish, *Macropodus chinensis* (BLOCH). Keijio Jour. Med., Vol. 5. No. 3. pp. 178-183, 2 pl.

25. SIKAMA, Y. 1938: On a new species of *Henneguya* found in a fresh water fish in China. Shanghai Sci. Inst. Vol. 4, 135-138, 1 pl.

PLATE I

Figs. 1-3. -*Myxidium enchelypterygii*; Figs. 1 and 2, front and side views of Delafield's haematoxylin-eosin stained spore. Figs. 3, a fresh spore.

Figs. 4-5. -*Myxidium matsuii*; Figs. 4 and 5, front and side views of Delafield's haematoxylin-eosin stained spore.

Figs. 6-7. -*Myxosoma dermatobia*; Figs. 6 and 7, front and side views of preserved spores.

Figs. 8-10. -*Myxosoma pseudorasborae*; Figs, 8-10, front, side and end views of fresh spores.

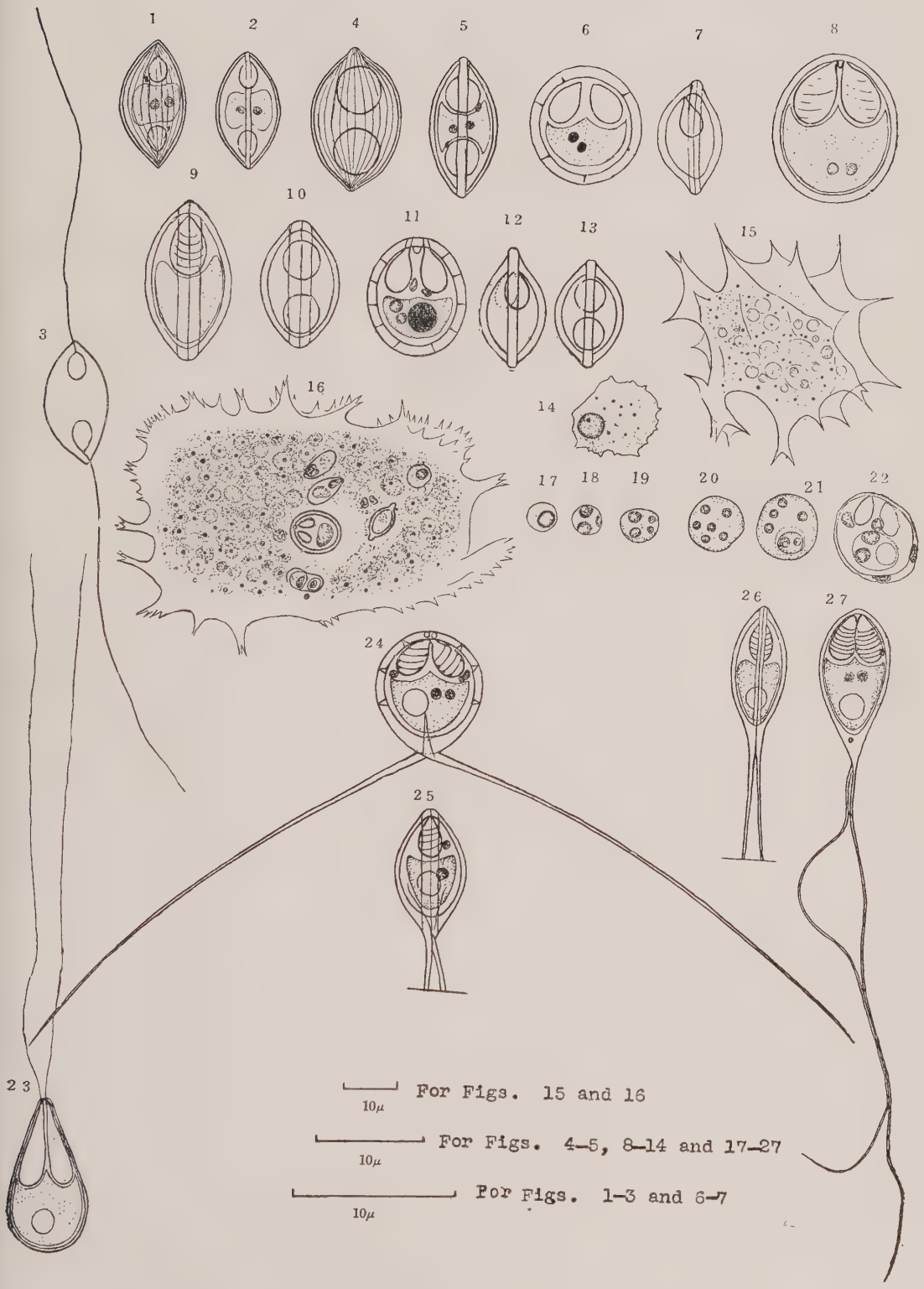
Figs. 11-22. -*Myxobolus acanthogobii*; Figs. 11-13, front, side and end views of preserved spores; Figs. 14-16, young trophozoites from the section preparation of brain of *Acanthogobius flavimanus*, stained with Delafield's haematoxylin-eosin; Figs. 14, the youngest trophozoite; Figs. 15, young trophozoite, the ectoplasm and endplasm are clearly diffrentiated; Figs. 16, young trophozoite, a few spores are formed in the endoplasm. Figs. 17-22, showing the spore formation, from the section preparation of the torophozoite. stained with Delafied's haematoxylin-eosin; Figs. 17 sporont; Figs. 18, three nuclear stage; Figs. 19, four nuclear stage; 20 and 21, six nuclear stage; Figs. Figs. 22, young sporoblast.

Figs. 23. -*Myxobolus koi*; Figs. 23, front view of fresh spore.

Figs. 24-25. -*Henneguya miyazakii*; Figs. 24 and 25, front and side views of Delafield's haematoxylin-eosin stained spores.

Figs. 26-27. -*Henneguya tridentigeri*; Figs. 26 and 27, side and front views of spores, stained with Delafield's haematoxylin-eosin.

PL. I.



10μ For Figs. 15 and 16

10μ For Figs. 4–5, 8–14 and 17–27

10μ For Figs. 1–3 and 6–7

PLATE II

Figs. 28-38. *-Trigonosporous acanthogobii*; Figs. 28, front view of Delafield's haematoxylin-eosin stained spore; Figs. 29, a spore viewed from the posterior end, preserved spore; Figs. 30-38, showing the spore formation, from the smear and section preparations, stained with Delafield's haematoxylin-eosin; Figs. 30, sporont; Figs. 31, two nuclear stage of the sporont, formed by first hetero polar division; Figs. 32-35, three, four, five and seven nuclear stages of the sporont; Figs. 36, young pansporoblast; Figs. 37, a pansporoblast at somewhat advanced stage in development; PC, pole capsule forming cells; shell forming nuclei; Spp, sporoplasms; Figs. 38, a pansporoblast at the more advanced stage in development; I. V, iodophile vacuole; PCN, pole capsule forming nuclei; PN; pansporoblast nucleus; SN, shell forming nuclei; SP. N, sporoplasm nuclei.

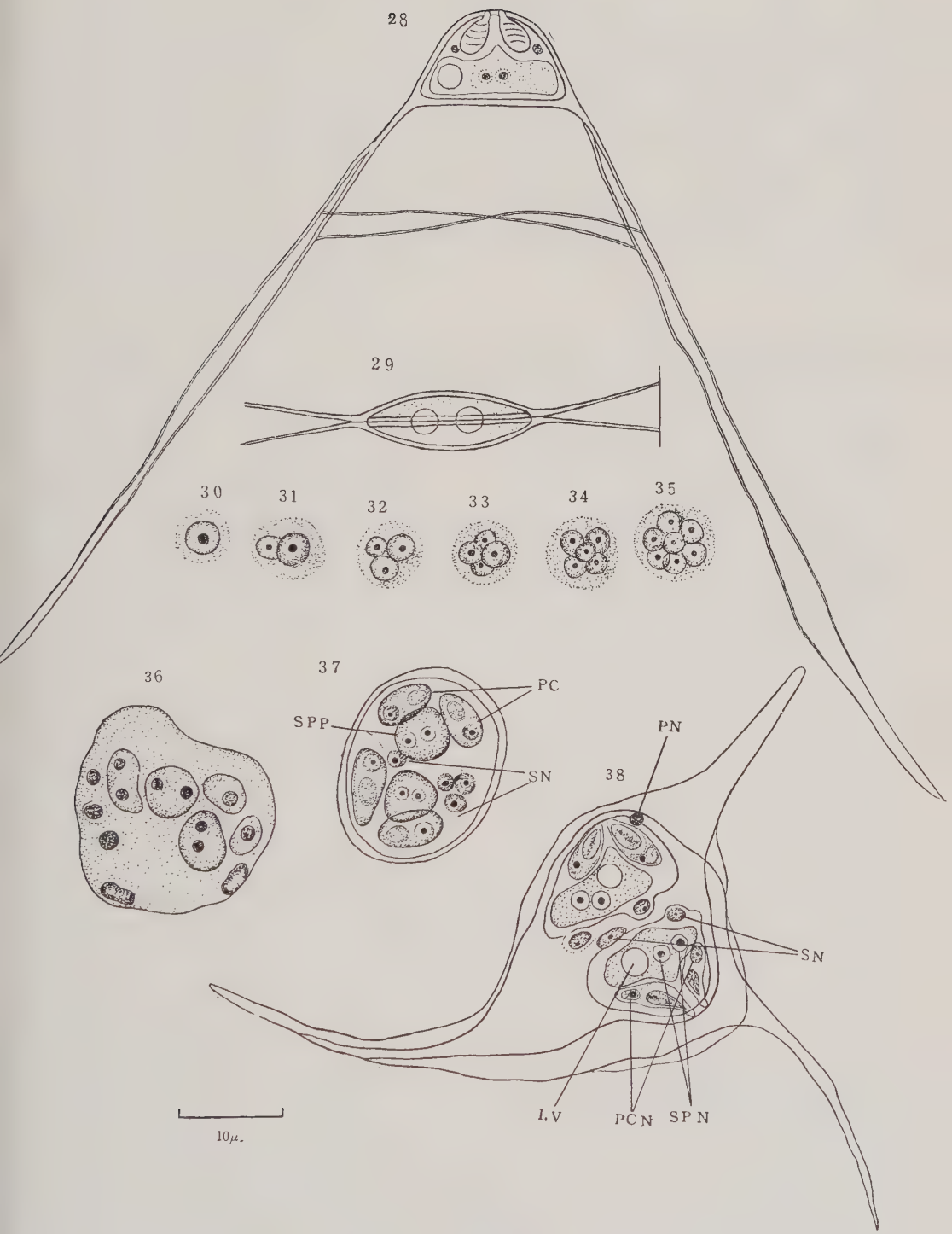

28
29
30
31
32
33
34
35
36
37
PC
SPP
SN
38
PN
SN
I.V
PCN
SPN
10μ.

PLATE III

Figs. 39. -A cyst of *Myxidium enchelypeterygii* on a dorsal fin of *Anguilla japonica* in section, stained with Delafield's haematoxylin-eosin.

Figs. 40-42. -*Myxidium matsuii*; Figs. 40, *Anguilla japonica*, showing the typical appearance due to the heavy infection of the skin by *Myxidium matsuii*; Figs. 41. showing the disintergrated skin of the host fish in section, stained with Delafield's haematoxylin-eosin; BC, blood cells; Cu, cutis; Cy, the ruptured cyst; EP, the ruptured epithelial tissue of the host's skin; Figs. 42, a young trophozoite from the section preparation of the skin of *Anguilla japonica* infected with *Myxidium matsuii*, stained with Delafiel's haematoxylin-eosin; Fb, fibroblasts; Ect, ectoplasm of the torophozoite; Spr. sporont.

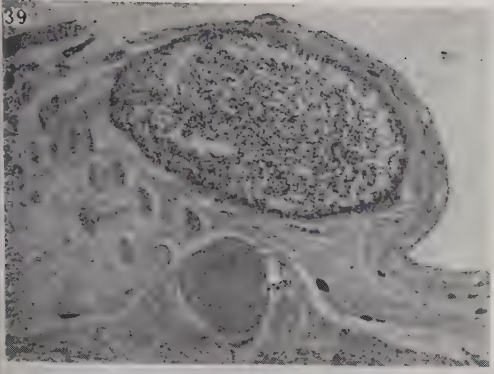
Figs. 43. -Showing the skin of *Anguilla japonica* with crowded cysts of *Myxosoma dermatobia*.

Figs. 44. -Sowing a single cyst of *Myxosoma dermatobia* in section, parallel section to the long-axis of the host body, stained with Delafield's haematoxylin-eosin.

Figs. 45. -Cross section of body muscle of *Pseudorasbora parba*, showing the cyst of *Myxosoma pseudorasborae*, stained with safranin-Delafield's haematoxylin.

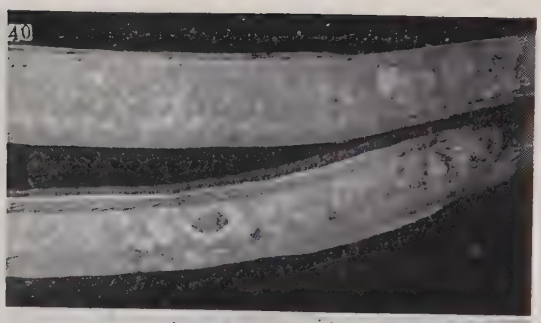
Figs. 46. -*Acanthogobius flavimanus*, showing the swelling of the periphery of the eye due to a heavy infection by *Myxobolus acanthogobii*.

39



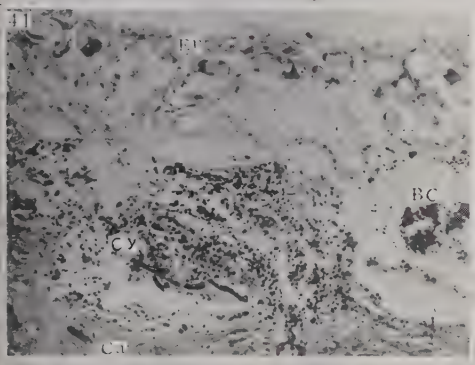
0.2mm

40



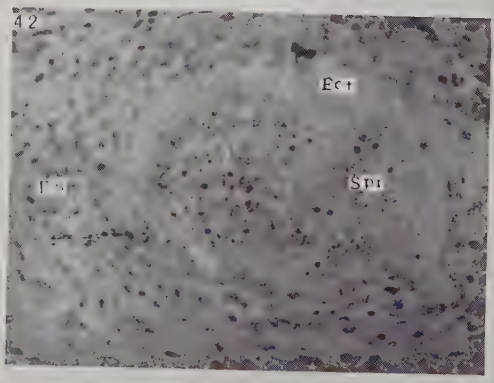
2 cm

41



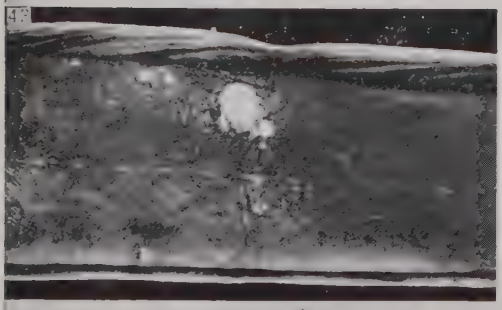
0.1mm

42



0.1mm

43

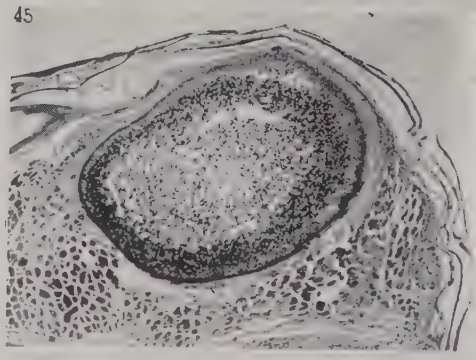


2 cm

44

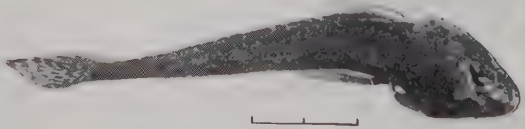
0.2mm

45



1mm

46



2cm

PLATE IV

Figs. 47-49.—*Myxobolus acanthogobii*; Figs. 47, Cross-section of *Acanthogobius flavimanus* through the orbital part, stained with Delafield's haematoxylin-eosin; Tro, trophozoite; M. ma, *Musculus masseter*; Figs. 48, Showing the torophozoites in section, cross section of olfactory nerve of the host fish, stained with Delafield's haematoxylin-eosin; N. ol, *Nervus olfactorii*; Figs. 49, Cross-section of the brain of the host fish, stained with Delafield's haematoxylin-eosin; BC, blood capillaries; T. op, *Tectum opticum*; Tro. trophozoite; V. cere, *Valvula cerebelli*.

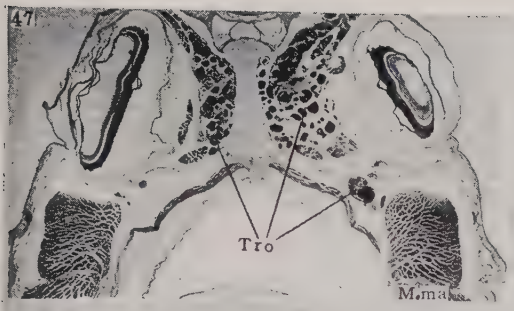
Figs. 50.—*Chaenogobius annularis annularis*, with cysts of *Henneguya miyazakii*.

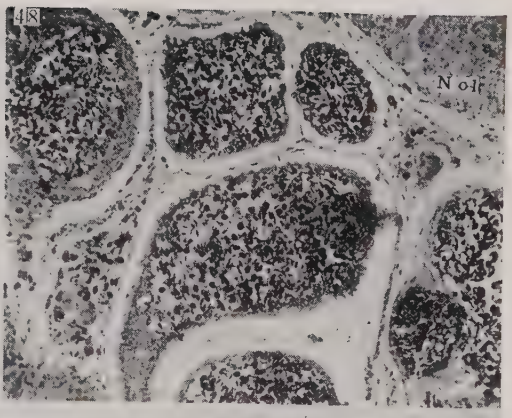
Figs. 51.—Cross-section of the integument of *Chaenogobius annularis annularis*, showing the cyst of *Henneguya miyazakii*, stained with safranin-Delafield's haematoxylin; Cu, cutis; Ep, epithelium; Sbc, subcutis; Sc, scale.

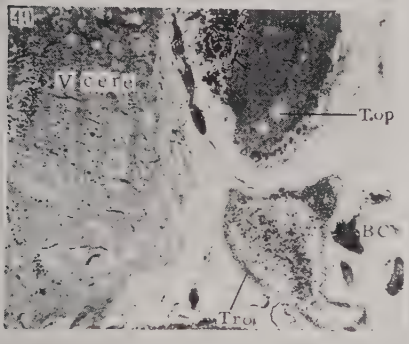
Figs. 52.—A gill of *Acanthogobius flavimanus* with cysts of *Henneguya tridentigeri* and *Trigonosporous acanthogobii*.

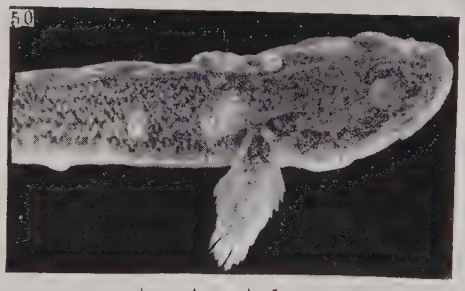
Figs. 53.—Showing the cysts of *Henneguya tridentigeri* and *Trigonosporous acanthogobii* in parallel section to the gill filaments, stsined with safranin Delafield's haematoxylin; 1. two cysts of *Henneguya acanthogobii*; 2. portion of section of a cyst of *Trigonosporous acanthogobii*.

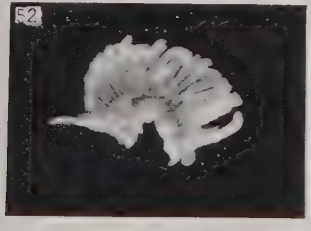
Figs. 54.—Part of a section of the cyst of *Trigonosporous acanthogbii*, stained witn Delafield's haematoxylin-eosin; CT, connective tissue of the gill filament; Ect, ectoplasm of the cyst; End, endoplasm of the cyst; Ep, epithelium of the gill filament; M, mucous cell of the gill filament; RF, respiratory filament; Sp, Spore.

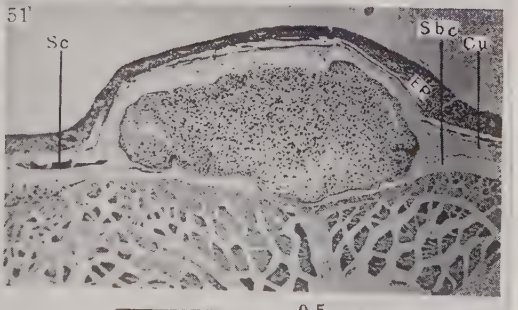

47
Tro
M.ma
1 mm

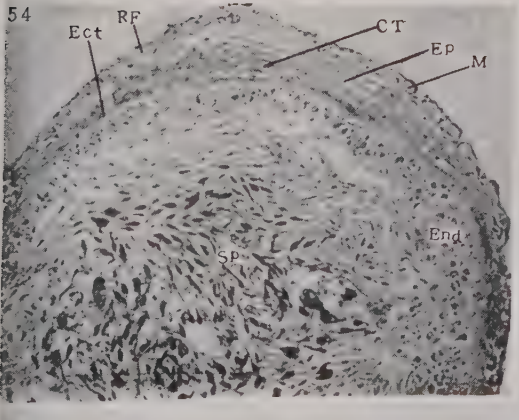

48
Not
0.1 mm

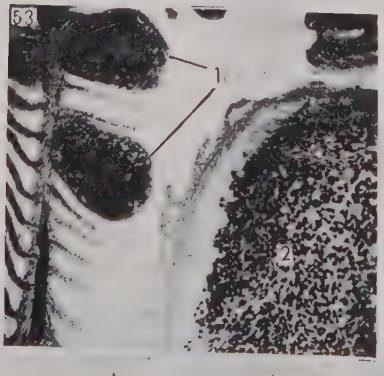

49
Viscera
Top
BC
Tro
0.2 mm


50
1 cm


52
2 mm


51
Sc
Sbc
Cu
FP
0.5 mm


54
Ect
RF
CT
Ep
M
End
SP
0.1 mm


53
1
2
0.2 mm

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

ON TWO RARE SPECIES OF PSALIDOPODIDAE AND NEPHROPSIDAE

Itsuo Kubo

(Received on Sept. 15, 1952)

In addition to descriptions of external characters, some descriptions of stomodaeal ossifications and discussions for taxonomy of two rare forms of prawns, *Psalidopus japonensis* sp. nov. and *Enoplometopus occidentalis* (Randall), belonging to Psalidopodidae and Nephropsidae respectively are given in this article, *Psalidopus japonensis*, which is taken from deep-waters off Kii peninsula, is new to science. This species may be regarded as third one of this genus, since two species, *P. huxleyi* Wood-Mason and Alcock and *P. spiniventris* Wood-Mason and Alcock have been known from Indian Ocean only. *Enoplometopus occidentalis* is secured from shallow waters off the same peninsula given above. This may be referred to a new record for the geographical distribution of this species.

Especial thanks are due Dr. H. Utinomi of the Seto Marine Biological Laboratory of the Kyoto University and Mr. Genkichi Abe for their kindness placing the specimens of these interesting species at my disposal. Also I wish to express herewith my sincere gratitude to the Ministry of Education, by which the present study has undergone the financial aid.

Psalidopus japonensis sp. nov.

(New Japanese name: Igaguri-ebi)

(Pl. V; Figs. 1-2)

Shell rather hard, tomentous and hispid. Rostrum well developed, strongly recurved upwards, about 1.6 times as long as carapace, armed with 10 spines on upper border, 11 ones on lower one, and about 20 spines on lateral border. Carapace furnished with a conspicuous spine on post-orbital edge, a prominent one a little below infra-orbital angle, 2 large and small branchiostegal spines, and 4 pterygostomian ones different in size. Moreover it also provided with median, dorso-lateral, infra-lateral rows of spines, and a series of spines arranged along upper boundary of branchial region; median row made up of 12 spines in female, 13 ones in male, a conical tubercle occurs just in front of posterior rim of carapace between the last spine of median row and posterior rim of carapace in both sexes; dorso-lateral row runs backwards from the post-orbital spines,

having 14 spines except post-orbital one on right side, 18 ones on left side; infra-lateral row follows to large branchiostegal spine, composed of 14 and 15 spines except branchiostegal one on right and left sides respectively. Last 2 thoracic sterna have a median acicula respectively. Last thoracic sternum provided on its posterior rim with a triangular upheaval abruptly rounded at apex and directing antero-ventrally.

Abdomen dorsally somewhat carinated; dorso-median carina has 4 spines[a] in first segment in female, 3 ones[a] in male, 6[b] and 5[b] ones in second segment of female and male respectively; 8 spines in third segment of female, 7 ones in male, 6 spines in fourth segment of female, 7 in male, 3 spines in fifth segment of female, 2 ones in male, 5 spines[c] in sixth segment of female, 4 ones[c] in male. Abdominal pleurae from first to sixth armed on lower border with 4, 8, 5, 5, and 5 acciculae and 4, 5, 4, 4, and 3 in female and male respectively.

Each pleonic somite, except telson, has an acicula on sternum, all the acciculae of anterior 5 somites set at middle, directing downwards, but the acicula of sixth somite is placed at middle of posterior border of the somite and directed posteriorly.

Antennular peduncle subcylindrical, made up of three joints. Basal joint about equal in length to the two remaining joints taken together, bears a longitudinal elevation on infero-internal edge and a digitate scale-like process on outer basal border; the longitudinal elevation has, at some distance from its apex, an acicular spine directing antero-medially; the scale-like process nearly reaching to distal rim of the joint on which it lies, bearing a large, shaply pointed apical spine. Intermediate and last joints subequal in length. Last joint has an acute spine near middle of outer margin (Fig. 1, *A*); flagella subequal in size, outer flagellum about 1.4 times as long as carapace, the other one a little longer than outer one.

Antennal peduncle carries three acicular spines on distal outer border of second segment. Antennal scale oblong, four times as long as wide, about 0.8 times the length of carapace, having nearly straight outer edge, has an acut stout disto-lateral spine which is greatly exceeded by rather broadly rounded anterior margin, fringed with spinules and hairs on outer edge as shown in Fig. 1, *B*.

Mandible distinctly divided into well calcified molar and incisor processes bearing a palp. Molar process square-prism-shaped, furnished with thickly set

(a) anterior two of them are small in size; (b) last spine is minute; (c) last spine is large.

rather short hairs on chewing edges. The other process large-spine-shaped, sharply pointed at tip, as long as molar process. Palp two-segmented, basal segment subequall to incisor one in length, distal segment somewhat shorter than basal one, with thickly set rather long hairs on apical and outer edges (Fig. 1, *C*). Maxillula comprises inner and outer laciniae and palp; palp without apical lobe, much smaller than those laciniae (Fig. 1, *D*). Maxilla has proximal and distal laciniae. Proximal lacinia thrice as wide as long, minutely produced at anterodistal angle, fringed on inner and posterior border with long hairs. Distal lacinia divided into 2 anterior larger posterior smaller lobes, anterior lobe about twice as large as the other one. Endopodite unsegmented (Fig. 1, *E*). First maxilliped composed of proximal, and distal laciniae, endopodite, and exopodite as is the case with maxilla. Distal lacinia proximally followed by a narrow lobe. Endopodite not segmented. Exopodite tapering, short, abruptly incurved (Fig. 1, *F*). Second maxilliped inverted L-shaped. Third maxilliped pediform, extending as far as the level of the base of antero-lateral spine of antennal scale, five-segmented; first and second segments short, third segment 4 times the length of second one when measured on outer edge, fourth one subequl in length to third one, last segment about 2.3 times as long as fourth one.

First leg reaches to about middle of antennal scale; merus 5 and half times as long as wide measured near distal end, armed with a large spine on distal upper border and about 7 small spines on distal half of dorsal edge; carpus short, runs into a large acut spine on distal uper rim, furnished with about 7 small spines on upper surface and a small one on distal dorso-lateral edge; propodus subcylindrical, about 2 and half times as long as wide, somewhat shorter than merus, about a quarter the length of carapace, armed with several spinules on proximal upper border; fingers about 0.7 times as long as propodus, having 13 teeth on distal half of cutting edge (Fig. 1, *G*). Second leg subequal in length to first one, but slenderer than first one; merus about 6 times as long as wide, ends in an acute spine on distal upper border; carpus about 0.8 times the length of merus; propodus somewhat less than carpus in length; dactylus replaced by a pencil of long setae, the pencil supported on apex of propodus, about 0.8 times as long as the segment on which it lies (Fig. 1, *H*). Third leg slender, reaching beyond tip of disto-lateral spine of antennal scale by about half of dactylus; merus armed with many spines especially on ventral border; carpus short, provided with 3 spines on distal edge; propodus somewhat more than thrice the length of carpus, carrying a pair of long spinous setae on disto-ventral border; dactylus about 0.4 times as long as propodus, sharply pointed

at tip, more or less recurved backwards (Fig. 1, *I*). Last two legs closely resemble third leg in all respects except proportional ones. Fourth leg streches to the same level of third leg, ischium, merus, carpus, propodus, and dactylus measure 6.5, 12.0, 3.5, 9.5, 3.8 mm respectively. Fifth leg longest of all legs, extending beyond apex of antennal scale by entire dactylus, measuring each segment from ischium to dactylus 6.5, 12.5, 5.3, 16.1, 4.5 mm respectively.

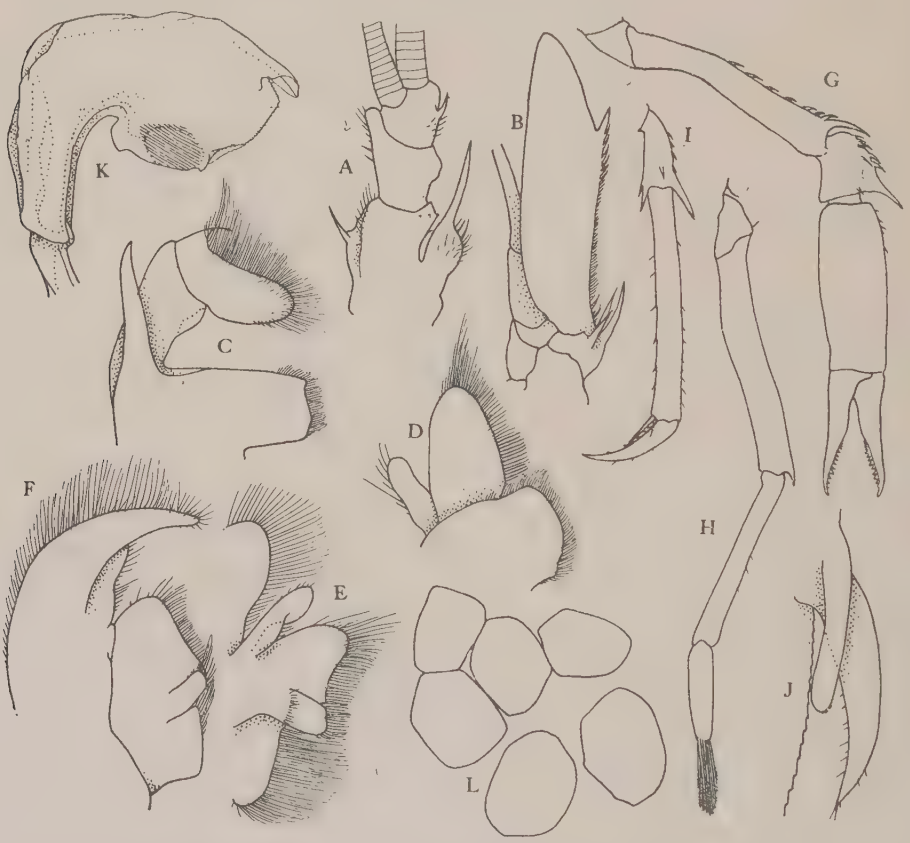


Fig. 1. Bodily parts of a male of *Psalidopus japonensis* sp. nov. *A*, upper aspect of antennular peduncle, × 4.5; *B*, antennal scale and others, × 2.5; *C*, mandible, × 14; *D*, maxillula, × 7; *E*, maxilla, × 7; *F*, first maxilliped, × 7; *G*, outer aspect of first pereiopod, × 3; *H*, outer view of second pereiopod, × 3; *I*, distal three joints of third leg, × 3; *J*, a part of endopodite of second pleopod of male with appendixes interna and masculina, × 10; *K*, stomach without posterior part, × 4.5; *L*, ovarian eggs, × 4.5.

Second pleopod carries an appendix interna and an appendix masculina on inner border of endopodite; appendix interna bar-shaped; appendix masculina tapering, distally curves downwards, much larger than appendix interna (Fig. 1, *J*).

Uropods a little longer than telson; armed with a spine on outer border of basal segment; outer uropod with nearly straight outer border fringed with thickly set spinules and a small disto-lateral spine.

Female closely resembles male in general feature, notwithstanding the following rspects are found different. Spines set on areas between rows of spines lying on carapace and pleon are somewhat more than those of male in number. Each acicula of last 2 thoracic sterna and abdominal segments from first to fifth is absent. The plate developed on posterior border of last thoracic sternum semicircular in outline, much wider than that of male. Endopodite of second pleopod uniramose as lacking appendix masculina.

Stomodaeal apparatus simple, no ossifications found to exist. Stomach is a specious bag, turns rightangularly backwards in posterior moiety, has an expansion on ventral wall. This expansion striated in posterior half with many slightly oblique longitudinal raw of thickly set yellowish fine short hairs on inner surface (Fig. 1, *K*). Posterior parts of stomach lost due to bad preservation.

Ovarian eggs are 48 in total number, measure about 3.3 × 2.4 mm (Fig. 1, *L*). Dimensions of bodily parts of 2 specimens dealt with are given in Table 1.

Table 1. Dimensions in mm of bodily parts of *Psalidopus japonensis*. BL, body-length; CL, carapecial length; OU, length of outer uropod except basal sement; RL, rostral length; SPS, length of sixth pleonic segment; TL, length of telson.

No.	Sex	BL	CL	RL	SPS	TL	OU
1	♀	94	29	46	12.0	14	14.0
2	♂	78	23	—	9.5	14	15.6

Colour: Ivory-white in alcohol.

Type-locality and others: Off Daiō-zaki, Kii Peninsula, taken Mr. Genkichi Abe at a depth of ca. 530 m by a deep-sea trawl net on April 25, 1943.

Holotype: Female, 94 mm in body-length.

Paratype: Male, 78 mm long with rostrum with about distal half broken off.

The species newly erected herewith closely resembles the two known species, namely *Psalidopus huxleyi* Wood-Mason and Alcock and *P. spiniventris* Wood-Mason and Alcock. But it is easily recognizable from them by the evidenses given in the following lines. First of all, the new species differs greatly from *P. huxleyi* and *P. spiniventris* in form of rostrum, especially in curvature

of, as shown in Fig. 2, and Plate V. The rostrum of the present species is much more strongly recurves upwards in comparison to that of two known species (Fig. 2, b, c, d). Telson does not reach to tip of uropods in *P. japonensis*, but that of the known species is surpassing distal extremity of the uropods according to the Fig. 21 of ALCOCK (1902), and to the Fig. 1 of WOOD-MASON and ALCOCE's Pl. 14 (1892). The spines of the median row of carapace is more or less greater in number comparing to those of the two known Indian species. Also the spines set on dorsal surface of the telson of *P. japonensis* are much larger in size than those of the tow forms in question.

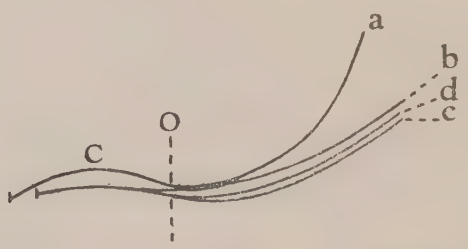


Fig. 2. Showing curvature of rostrum of *Psalidopus*-species. Lines are showing upper border of rostrum and carapace. *a*, *P. japonensis* sp. nov. based on the photograph given in the Plate V of this journal; *b*, *P. spiniventris* taken from the Fig. 21 of ALCOCK, 1902; *C*, showing carapacial part; *c*, *P. huxleyi* based on the Pl. 14, fig. 1 of WOOD-MASON and ALCOCK, 1892; *d*, *P. huxleyi* based on the Pl. 16, fig. 47 of DE MAN, 1920; *o*, indicating the level of post orbital edge.

In addition to those given above, the uropods of the Japanese species appear to be shorter than those of the Indian ones. The proportion of length of the uropods to that of the sixth pleonic segment measured along the median line are 1.16 in the female and 1.47 in the male. Whilst those of the Indian forms are 1.41 in *P. huxleyi* and 1.75 in *P. spiniventris* due to figures of WOOD-MASON and ALCOCK, and ALCOCK, 1892, Pl. 14, fig. 1, and ALCOCK, 1902, fig. 21 respectively.

Finally the matters given below may be worthy of mentioning. The stomodaeal apparatus of the present species is showing close resemblance to that of Palaemonidae in general feature and in respect of utterly absence of ossifications. With regard to the fifth pereiopods, the ratio of length of the propodus to that of the carpus is 3 in both specimens at my disposal as is the case with ALCOCK's *P. spiniventris*. Also it is 3 in DE MAN's *P. huxleyi*, but 2 in WOOD-MASON and ALCOCK's one.

Enoplometopus occidentalis (Randall)

(New Japanese neme: Shyōgun-ebi)

(Pl. VI; Fig. 3)

Nephropus occidentalis, Randall, 1840 (1839?), p. 139.
Enoplometopus pictus, Miers, 1880, p. 380.
Enoplometopus occidentalis, Ortmann, 1897, p. 274; Rathbun, 1906, p. 900, Pl. 17, fig. 2; Barnard, 1934, p. 665; Bonde, 1935, p. 6; Holthuis, 1946, p. 47–79, Pl. 5, figs. a. c. f. i.

The specimen, on which the description and illustrations given later are based, is a male measuring 64.7 mm in body-length. It was taken from Taiji, Wakayama prefecture by Mr. Taira Horihata in March, 1952, and has been sent to the present Author for identification through Dr. H. Utinomi.

For Hawaiian specimen of this species, a valuable description and a splendid photograph have been afforded by Rathbun. Recently a very minute description and illustrations also have been done by Holthuis. The specimen at the author's disposal tallies well with those descriptions and illustrations in almost all important characteristics involving gonopod and mouth-parts, although some minute diffarences are found to exist on the third maxilliped and others. Accordingly it may be referred to the present species.

Some descriptions and illustrations of the present specimen may be given in the following lines supplementing those of Holthuis mainly in regard to the stomodaeal apparatus and colouration.

Antennular peduncle provided on distal inner corner and on a position a little anterior to middle of inner border of basal segment with a minute spine respectively; flagella rather long, inner flagellum 1.7 times as long as carapace, outer one somewhat thicker and shorter than the other one, about 1.1 times as long as carapace.

Mouth-parts closely resemble those of *Enoplometopus antillensis* Lütk (Holthuis, Pl. 6, figs. a–e) in general feature excepting mandibular palp. Last segment of the palp rather swollen instead of being slender in *E. antillensis*, about twice as long as wide measured at middle (Fig. 3, *B*). Third maxilliped 7-jointed; extending beyond tip of rostrum by last two segments; ischium and merus stout, ischium has a large acute spine on distal inner border, and on inner surface a median longitudinal elevation with about 18 small spines; merus armed with one antero-dorsal and two antero-ventral spines; last three segment rather slender, with no spines at all (Fig. 3, *C* and *D*).

All pereiopods stout; chelae of second and third pereiopods incomplete as having less developed fixed finger or disto-ventral projection of propodus. First

and second pereiopods streches beyond tip of rostrum by distal three joints respectively. Fifth one extending as far as eyes.

Stomodaeal ossifications: Cardiac ossicle large, bar-shaped, a little curved anteriorly toward both sides, about 4 times as long as wide (Fig. 3, *G*, a). Urocardiac ossicle somewhat elongated, pentagonal as being broadly angulated on anterior border, with each antero-lateral angle considerably produced sidewise, provided on inner surface close to distal extremity with a pair of large teeth ovoid and flatish on upper surface; teeth coloured yellowish orange (Fig. 3, *G*, b and *J*). Pterocardiac one small, triangular in shape, rather abruptly curves backwards in distal extremity (Fig. 3, *G*, c). Zygocardiac ossicle large, provided with the oblong teeth and a slender bar-shaped ossicle running downwards near antero-ventral angle (Fig. 3, *G*, d and *H*). Prezygo-cardiac one also bar-shaped, furnished on distal end with a large triangular swelling which bears three vestigial conical teeth on inner surface (Fig. 3, *G*, e, and *I*). Prepyloric

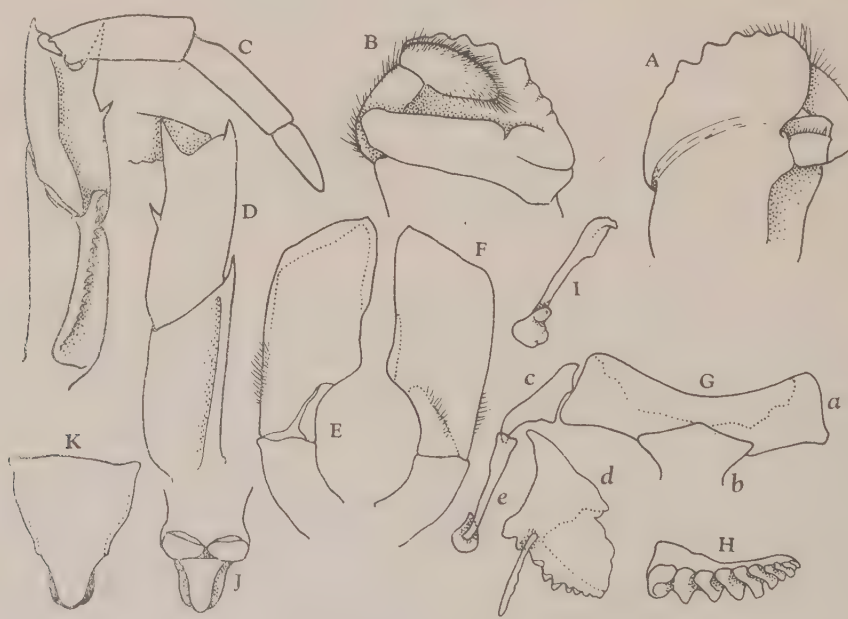


Fig. 3. Bodily parts of *Enoplometopus occidentalis* (RANDALL). *A*, outer view of mandible, × 7; *B*, inner aspct of mandible, × 7; *C*, inner aspect of third maxilliped, × 3; *D*, outer aspect of ischium and merus of third maxilliped, × 3; *E*, outer view of first pleopod of male, × 5; *F*, inner view of first pleopod of male × 5; *G*, stomodaeal ossifications (*a*, cardiac ossicle; *b*, anterior part of urocardiac ossicle; *c*, ptero-cardiac ossicle; *d*, zygocardiac ossicle; *e*, prezygocardiac ossicle), × 15; *H*, teeth of zygocardiac ossicle, × 20; *I*, prezygocardiac ossicle, × 20; *J*, ventral aspect of distal tooth of prepyloric ossicle and distal part of urocardiac one, × 20; *K*, outer aspect of prepyloric one, × 20.

ossicle triangular on dorsal aspect, as long as wide, convex on upper surface, ending into an apical tooth rounded on apex and stained with brown colour (Fig. 3, *K*).

Colour: All body and appendages are stained with light whitish chrom orange colour. Body marked with many spots of rather deep chrome orange colour. The spots measure about 2 mm in diameter, those of both sides of body are defined more clearly than those found on other parts of body. All spines lying on cephalo-thorax including rostrum and first chelipeds dyed deeply with chrom orange colour in basal part, and most of them are of white colour at tip. Chelae of first pereiopods with 3 bands of rather deep chrome orange colour, and tipped with the same colour. Exopodite of uropod has a semicircular mark of violet colour near its tip.

Dimensions of bodily parts in mm are as follows; Body-length except rostrum 64.7, lengths of carapace, abdomen, rostrum, and telson are 23.0, 41.7, 11.4, and 10.0 respectively, and those of pereiopods in order from first to fifth are 63.0, 42.3, 39.4, 36.4, 30.5.

Distribution: Natal coast (Barnard, 1934; Bonde and Marchand, 1935), Port Louis, Mauritius (Bouvier, 1910, '14, '15), Ambon, Banda Sea (Miers, 1880; Man, De, 1888, 1924; Ortmann, 1894; Holthuis, 1946), Banda (Balss, 1933), Hawaiian Islands (Kingsley, 1883, Rathbun, 1906).

Remarks: The present specimen differs from the descriptions and figures given by Holthuis in the following point only. The propodus of the third maxilliped of the specimen dealt with herein has no spines at all. But Holthuis describes for the segment in question of his specimen as it is having two spines on lower inner margin and a spine on antero-ventral one. According to the description made by Rathbun, the proportion of the body-length to carapecial length is 2.35 in the Hawaiian specimen. Whilst that of the present specimen is 2.81. But the proportion of the Hawaiian specimen seems to be based on a body-length except telson-length, since the proportion of the present specimen taken basing on body-length without telson is 2.37, which is almost equal to that of the Hawaiian specimen given above. Also it may be mentioned herewith that the proportion of length of telson to breadth of telson measured at middle is almost equal in both specimens examined by the present author and Holthuis as being 1.3 in the present specimen and 1.2 in the Holthuis specimen according to his illustration.

Lastly it may be noticed that the matters set forth in this paragraph have not been mounted on the description given by Holthuis. The basal segment of the antennal peduncle has a small but stout spine on distal-inner angle

and a spinule near middle of the inner border. The merus of the fourth pereiopod is provided with a distoventral spine as is the case with the second and third ones.

REFERENCES

The papers marked with an asterisk (*) were not available to the writer.

(1) ALCOCK, A. 1901. A descriptive catalogue of the Indian deepsea crustacea: Decapoda, Macrura, and Anomala in tne Indian Museum. Being a revised account of the deep-sea species collected by the Royal Indian Marine Survey Ship Investigator, pp. 1-286, i-iv, Pls. 1-3.

(2) ———. 1902. A naturalist in Indian Seas, pp. 1-318 (London).

(3) BARNARD, K. H. 1934. The lobster, *Enoplometopus occidentalis* RANDALL, in South Africa. Nature, London, Vol. 134, p. 665.

(4) BONDE, C. VON and J. M. MARCHAND. 1935. The natural history and utilization of the cape crawfish, Kreef, or spiny lobster, *Jasus* (*Palinurus*) *lalandii* (MILNE-EDWARDS) ORTMANN. Fish. Bull. Fish. Mar. Biol. Surv. South Africa, Vol. 1, pp. 1-55, Pls. 1-8, Maps 1-9.

(5) GURNEY, R. 1938. Larvae of decapod crustacea. Part V. Nephropsidea and Thalassinidea. Discovery Rep., Vol. 17, pp. 291-344, figs. 1-39.

(6) HOLTHUIS, L. B. 1946. Biological results of the Snellius Expedition. XIV. The Decapoda Macrura of the Snellius Expedition. 1, The Stenopodidae, Nephropsidae, Scyllaridae, and Palinuridae. Temminckia, Vol. 7, pp. 1-178, Pls. 1-11.

(7) MAN, J. G. DE. 1916. The Decapoda of the Siboga Expedition, Part III. Fam. Eryonidae, Palinuridae, Scyllaridae, and Nephropsidae. Siboga-Exped., Mono., 39a2, 1-122, Pls. 1-4.

(8) ———. 1920. The Decapoda of the Siboga Expedition, Part IV. Fam. Pasiphaeidae, Stylodactylidae, Hoplophoridae, Nematocarcinidae, Thalassocaridae, Pandalidae, Psalidopodidae, Gnathophyllidae, Processidae, Glyphocrangonidae and Crangonidae. Siboga-Exped., Mono., 39a3, pp. 1-318, Pls. 1-25.

(9) ———. 1922. The Decapoda of the Siboga Expedition. Part V. On a collection of macrurous decapod crustacea of the Siboga Expedition, chiefly Penaeidae and Alpheidae. Siboga Exped. Mono., 39a4.

(10) MIERS, E. J. 1880. On a collection of crustacea from the Malaysian region, Part III. Crustacea Anomura and Macrura (except Penaeidea). Ann. Mag. Nat. Hist., Ser. 5, Vol. 5, pp. 370-384, Pls. 14, 15.

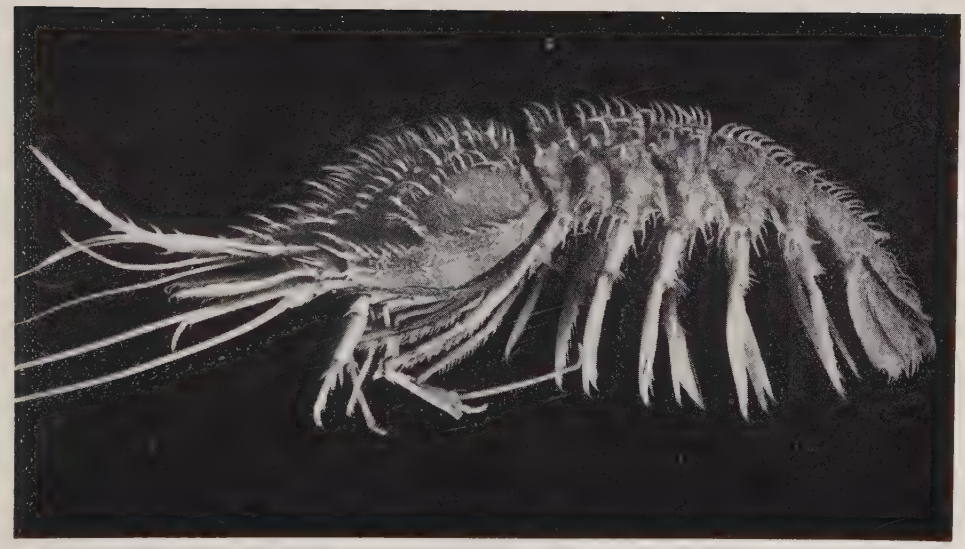
(11) ORTMANN, A. 1897. Carcinologische Studien. Zool. Jahrb. Syst., Vol. 10, pp. 258-372, Pl. 17.

(12)* RANDALL, J. W. 1839. catalogue of the Crustacea brought by Thomas NUTTALL and J. K. TOWNSEND, from the West coast of North America and the Sandwich Islands, with descriptions of such species as are apparently new, among which are included several species of different localities, previously existing in the collection of the academy. Journ. Acad. Nat. Sci. Philad., Vol. 8, Pt. 1, pp. 106-147, Pls. 3-7.

(13) RATHBUN, Mary J. 1906. The Brachyura and Macrura of the Hawaiian Islands. Bull. U. S. Fish Comm., Vol. 23, Part 3, pp. 827-930, Text-figs. 1-79, Pls. 3-24.

(14) WOOD-MASON and A. ALCOCK. 1892. Natural history notes from H. M. Indian Marine Survey Steamer "Investigator", commander R. F. HOSKYN, R. N., commanding. Ser. II, No. 1. On the results of deep-sea dreding during the season 1890-91. Ann. Mag. Nat. Hist., Vol. 19, Sixth Ser., pp. 265-275.

PLATE V



Psalidopus japonensis, sp. nov., female, 94 mm in body-length (29 mm in carapecial length).

PLATE VI

Enoplometopus occidentalis (RANDALL), male, 64.7 mm in body-length (23.0 mm in carapace-length).

Journal of the Tokyo University of Fisheries, Vol. 39, No. 1, 1952

ON THE BOTTOM CHARACTERS OF THE INSULAR SHELF AROUND HACHIJO ISLAND AND THE NEIGHBOURING BANKS.

Hiroshi Niino

(Received Oct. 10, 1952)

INTRODUCTION

The purpose of this article is to report on the geological, oceanographical, and topographical features, the marine deposits and fauna, in the vicinity of Hachijo Island, a small islet group lying off the Pacific side of Honshyu, Japan. These islets stretch in north to south direction and constitute a sort of Fuji volcanic chain. The banks and the islands are valuable fisheries grouud and also important in the solution of geological problems of the Japanese Island.

Dr. S. Hatai and Dr. K. Hidaka have succeeded in gathering some scientists who wishes to make scientific investigations at Hachijo Island. This group called "Hachijojima Sogo Kenkyu Iinkai" and aided by a grant from the ministry of Education. The writers are the member of this group and are engaged in this research.

During the year 1951 to 1952, the training ship Shinyomaru, Shunkotsumaru and Umitakamaru of the Tokyo University of Fisheries visited Hachijo Island for the purpose of discovery the new fisheries ground. The writer obtained many bottom samples and oceanagraphical data at the time of voyage.

The following descriptions are the resalts of the examination on the bottom materials dredged at the time of training voyage in the summer of 1951 by Shinyomaru and Shuokotsumaru.

PART. I.

MARINE FAUNA DREDGED FROM NEAR HACHIJO ISLAND

by

(Kotora Hatai, Hiroshi Niino and Tamio Kotaka)

The material comrising this part were dredged by H. Niino, Professor of the Tokyo University Fisheries during the late Summer of 1951, from a total of five stations in the sea offing Hachijo Island.

The material so far identified and their respective stations with latitude, longitude and depth in meters are given in the list of speics. From the list it may

at once be recognized that several fossil species of molluscs are intermingled. Besides the species which are common to Japan, several new species of Hawaiian affinity and many of fossil aspect characterize the dredged fauna; their descriptions and illustrations are presented herein.

The occurrence of *Tucetona hanzawai* (Nomura and Zinbo) and *Venericardia ryukyuensis* Nomura and Zinbo from Shinkurose together with many others of fossil aspect, suggests the existence of a submerged geological formation which may correspond in age to a formation with similar species in the Ryukyu Islands. If so, then on the northern side of Hachijo Island we may expect to find the northern extension of the Ogasawara Arc circumscribing in part, the Fuji Volcanic Chain to which Hachijo Island belongs.

The molluscs with fossil aspect but which are still persistent in the adjacent seas of Japan are in need of explanation. These shells may represent a formation still younger than the one inferred from the aforementioned fossils. That is to say, a submerged raised beach deposit or some Pleistocene formation built up of incoherent material as coarse sand. However, since dredge operations are expected to be continued, further comments are here withheld. Since there still remain some indetermined shells which have fossil aspect from Shinkurose, another paper will be devoted to summarize the results of study on the dredged marine fauna.

This work was financed by a grant from the ministry of Education and is one of the projects initiated by the Hachijo Island Investigation Committee under the auspices of the Research Council of Japan, to whom our thanks are due.

GASTROPODA

Bursa bufonia (Gmelin)

A single specimen with part of the dried animal attached to the inside is found in the collection. The shell is not in natural coloration and so it seems that the shell may have been occupied by some hermit crab.

Station.-Shinkurose, St. 1. Lat. 33°30.30.0′N., Long. 140°07.5′E., 280 m.

Cavolina trispinosa (Blainville)

Six broken spines belonging to the named species were found intermingled in the coarse sand of Shinkurose. Of these spines two are badly faded and have the aspect of sossil rather than of Recent specimens.

Station--Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Clathrofenella reticulata (A. Adams)

The specimens of this species are all discolored, more or less water worn, and fractured at their apical region. None appear to be Recent.

Station.-Shinkurose, 8t. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Collisella heroldi (Dunker)

A single immature specimen still retaining the original coloration along the margins was found intermingled in the sand dredged from the cited station. It was probably a species living along the rocky shores of the island, but which sank to its place of discovery after death.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Conus sp. (Fig. 1).

A single high spired *Conus* with equispaced tubercles sculpturing the body whorl which is yellowish white with several splashes of a brownish red color is in the collection. So far as study has been carried, species with which the present one could be identified have not been found. This may represent a new species.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m.

Conus sp. (Fig. 2).

An interesting *Conus* with rather high spire, light purple color with dots and splashes of brownish red on its surface and without sculpture except for about ten incremental lines at the base of body whorl. This species, which has part of the dried animal still attached also seems to represent a new species, but naming is withheld owing to the lack of literature.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Coralliophila euegniae (Bernardi)

A single specimen retaining both natural coloration and detail sculpture is represented in the collection. The shell is partially covered with adhering organisms and it seems that not much time has elapsed since the animal died.

Station.-Shinknrose, St. 1. Lat. 33°30.0′N., Long. 140°07.5′E., 280 m.

Margarites cf. *cincereus* (Couthouy)

Several discolored, water worn and fractured specimens were found in the coarse sand from the cited station. These, if really Recent, were either embedded in sediments or unoccupied by the animal for a rather long time. From their coloration and state of preservation it appears that they are fossil.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Mucronalia subulata (A. Adams)

The specimens are all fresh.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Ocenebra adunca (Sowerby)

A broken body whorl partially encrusted with a bryozoan, but agreeing in all observable features with the named species. This species is a common

shallow water animal which crawls on rocky environments. It was probably carried to its place of burrial after death.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E. 115 m.

Phalium sp.

A fragment of the body whorl with the varix attached. The retained coloration suggests that this fragment may be *Phalium strigatum* (Gmelin), a common shell in southwest Japan.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m.

Pseudoliotia micans (A. ADAMS)

Two specimens, one is faded and the other is a dead shell but with rather fresh appearance.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Tonna luteostoma KUSTER

A single immature specimen with the soft part retained at the time of collection is in the collection. The shell is thinner than the specimens commonly found strewn along the beaches of the Miura and Boso Peninsulas in the Kwanto Area. The color is dull compared with Recent specimens.

Station.-Shinkurese, St. 1. Lat. 33°30.′N., Long. 140°07.5′E., 280 m.

PELDCYPODA

Arca sp.

Two specimens of a young *Arca* measuring about 2 mm in length and length and retaining the detail sculpture were found intermingled in the coarse sand from the cited station. Their specific identification is at present doubtful.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Callista rosida (GOULD)

A single small right valve with the coloration faded and the antero-dorsal border pierced with a hole made by some carnivorous gastropod.

Station.-Hachijo-jima, St. 2. Lat. 33°08.5′N., Long. 139°45.9′E., 200 m.

Lima lima LINNAEUS

One small specimen with closed valves and a larger of the right valve. The large specimen has its inner and outer surfaces encrusted with bryozoa and the shell is water worn. The smaller specimen still retains the soft part. These are from Station 1. The broken and water worn faded right valve from Station 2, has encrusting bryozoa on its surfaces.

Stations.-Hachijo-jim, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m. Hacaijo-jima St. 2. Lat 33°08.5′N., Long. 139°45.0′E 200 m.

Meretrix sp.

An immature, water worn, faded left valve of *Meretrix*.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m.

Nipponolimopsis cf. *nipponica* (Yokoyama)

A small discolored valve closely resembling the named species is in the collection. The valve is bored by a small hole, probably of some carnivorous gastropod.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N, Long. 140°10.0′E., 290 m.

Notostrea musashiana (Yokoyama)

Two small lower valves, one is faded to yellowish-brown and is partially filled with scoria sand, and the other is fresh but water worn. The faded valve is probably a fossil.

Station.-Hachijo-jima, St. 2, Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

Pseudochama sp.

A single lower valve. It is thin, pale inside and of yellowish color outside. It is elongate oval in shape, shallow ventrally and deep dorsally. This lone specimen probably represents a new species but owing to the upper valve having been lost, naming is reserved for another opportunity.

Station.-Hachijo-jima, St. 2. Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

Spondylus cf. *cruentus* Lischke

A large lower valve; it is bady water worn, faded, encrusted with bryoza, annelid tubes, calcareous algae and with a solitary coral attached near the umbonal region, the solitary coral is also encrusted by a water worn bryozoan species.

Station.-Hachijo-jima, St. 1. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m.

Spondylus sp.

Four isolated valves of *Spondylus* are in the collection. Two retain their original coloration and detail sculpture but are immature.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m.

Spondylus sp.

A fragment of the upper valve.

Station.-Hachijo-jima, St. 2. Lat. 33°08.5′E., Long. 139°45.0′E., 200 m.

Tucetona hanzawai (Nomura and Zinbo) (Figs. 3, 4).

The single water worn specimen measuring about 15.5 mm in height, 16.5 mm in length and referable to the named fossil species was found in the collection.

Tucetona hanzawai (Nomura and Zinbo) (S. Nomura and N. Zinbo, 1934, Sci. Rep. Tohoku Imp. Univ., Ser. 2, vol. 16, no. 2, p. 152, pl. 5, figs. 3 a, b) is an extinct species, known only from the socalled Pleistocene Ryukyu limestone

of Kikai-jima and also form Okinawa (T. Habe, 1951, Genera of Japanese Shells, no. 1, p. 42). The occurence of this species in the marine fauua of the environs of Hachijo-jima is interesting because it may suggest the occurrence of a submerged formation corresponding in age to the Ryukyu limestone.

Station.-Shinkurose, St. 1. Lat. 33°30.0′N., Long. 140°07.5′E., 280 m.

Tucetona sp.

A single left valve. It is well preserved, lacks the original coloration; orbicular in outline, inflated, provided with about 30 radial ribs including the acessory ones at the anterior and posterior extremities.

This lone shell resembles *Glycymeris pectinata* Nomura and Zinbo (S. Nomura and N. Zinbo, 1934, Sci. Rep. Tohoku Imp. Univ., Ser. 2, vol. 16, no. 2, p. 112, pl. 5, fis. 1a, 1b; not of Lamarck) from the Pleistocene Ryukyu limestone of Kikai-jima.

Station.-Hachijo-jima, St. 2. Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

Vasticardium arencolum (Reeve)

Two isolated left valves. The smaller of the two retains the original coloration and is not water worn, while the larger, which is faded and with a

Fig. 1. *Conus* sp. Natural size; Fig. 2. *Conus* sp. Natural size; Figs. 3, 4. *Tucetona hanzawai* (Nomura and Zinbo). Natural size; Figs. 5, 6. *Venericardia ryukyuensis* Nomura and Zinbo. Figs. 7, 8. *Barbatia hachijojimensis* Hatai, Niino and Kotaka, n. sp. Natural size; Figs. 9, 10. *Arca mauria takii* Hatai, Niino and Kotaka, n. sp. Natural size. Figs. 11, 12. *Tucetona shinkurosensis* Hatai, Niino and Kotaka, n. sp. Natural size.

balanid, bryozoan and annelid tubes attached to its surface is somewhat water worn.

Station.-Sninkurose, St. 1. Lat. 33°30.0′N., Long. 140°07.5′E., 280 m. Hachijo-jima, St. 2 Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

Vasticardinm sp.

A fragment of a fresh right valve. The fragmen resembles the above mentioned species.

Station.-Shinkurose, St. 1 Lat, 33°30.0′N., Long. 140°07.5′E., 280 m.

Venericardia ryukyuensis Nomura and Zinbo. (Figs. 5, 6).

Three discolored specimens are referred to *Venericardia rykyuensis* Nomura and Zinbo (1936, Sci. Rep. Tohoku Imp. Univ., Ser. 2, Vol. 18, No. 3, p. 240, pl. 11, figs. 7a, b, 8a, b), described by those authors from the Neogene Shimajiri formation of Okinawa Island. One of the spccimens is a left valve filled with compact volcanic sand and the surface surface sculpture is badly water worn. The other two specimens are also water worn but without filling of sediment.

Statian.-Shinkurose, St. 3. Lat. 33°24.5N., Long. 140°10.0′E., 290 m.

Venus (*Chione*) *chlorotica* Philippi

A broken, upper half of the right valve is represented in the collection. The shell is faded, water worn with adhering bryozoa and annelid tubes on the surface and inside. This may be a fossil.

Station.-Shinkurose, St. 1. Lat. 33°33.0′N., Long. 140°07.5′E., 280 m.

Venus sp.

A fragment of the lower half of the shell. The fragment is water worn, faded and partially encrusted with water worn bryozoans on both surfaces. The sculpture of the shell resembles *Venus toaeuma* Gould, a common Recent shell of southwest Japan, but is thinner.

Station.-Hachijo-jima, St. 2. Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

SCAPHOPODA

Cadulus sp.

A single immature specimen.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

Descriptions of New Species

Barbatia hachijojimensis Hatai, Niino and Kotaka, n. sp.

(Figs. 7, 8)

Shell of moderate size, 31 mm long, 14.5 mm high and about 12 mm in depth of closed valves; elongate-ovate, rather compressed, with a thin perio-

stracum. Hinge almast straight, ventral margin slightly constricted a ilttle anterior to middle, a slight depression extends there from dorsally to umbo. Anterior side abruptly rounded, the posterior side sharply protruding, forming obtuse angle with hinge-line; this part provided with ribs stronger than elsewhere. Outer surface of shell provided with fine radiating ribs, numbering about 10 per 5 mm, crossed by growth lines which cut the radials up into granules or rectangular squares, making surface appear cancellated; posterior lateral part with about seven cancellated radiating ribs, which are somewhat stronger than those adjacent to them ventrally. Color reddish brown with yellosh gray transverse bands on surface; inner surface colored light reddish purple becoming lighter ventrally. Ventral border without gapping of valves. Ligamental area narrow, with about three grove, the anterior part about one-fifth of the length of hinge-line. Hinge-line with 11 anterior and 29 posterior teeth, which decrease in size regularly from the sides inwards.

Remarks.—This species resembles *Barbatia parva* (Sowerby), figured by Reeve (1844, Conch. Icon., pl. 17, fig. 110) and by Kobelt (in Martini and Chemnitz, 1891, Conch. Cab., vol. 8, p. 197, pl. 47, fig. 7), but can be distinguished therefrom by the much larger size (*B. parva*, measures only 15–16 mm in length), less inflated and lower beak and by the less constricted ventral margin. *Barbatia* (*Abarbatia*) *molokaia* Dall, Bartsch and Rheder (1938, Bernice P. Bishop Mus., Bull. 153, p. 32, pl. 5, figs. 7–10, text-fig. 11) from off the southern coast of Molekai in the Hawaiian Islands, also resembles the present species in general features, but can be distinguished by the details of surface sculpture.

The present new species is considered to be the northern representative of the small-sized tropical species.

Station.-Hachijo-jima, St. 1. Lat. 33°06.5′N., Long. 139°52.6′E., 115 m. Holotype). Hachijo-jima, St. 2. Lat. 33°08.5′N., Long. 139°45.0′E., 200 m. (Paratype).

Arca mauria takii Hatai, Niino and Kotaka, n. subsp.
(Figs. 9, 10)

The single right valve at hand agrees with the named species in the type of coloration, shape of the shell general features of the surface sculpture and also in size. This valve can be distinguished from *Arca mauia* (Dall, Bartsch and Rheder) (1938. Bernice P. Bishop Mus., Bull. 153, p. 12, pl. 1, figs. 1–4, text-fig. 2) by having about 40 instead of 32 teeth and the size is a little larger,

being 21 mm in length and 10 mm in height and about 5 mm in depth. These differences are considered worthy of subspecific separation of the present specimen from Dall, Bartsch and Rheder's species.

The name is given to Dr. Iwao Taki of the Tokyo Science Museum in recognition of his work on the molluscan fauna of Hachijo Island.

Station.-Hachijo-jima, St. 2.-Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.

Tucetona shinkurosensis Hatai, Niino and Kotaka, n. sp.
(Figs. 11, 12)

Shell small, inflated, subcircular, longer than high, anterior part shorter than posterior; test solid. Beak small. Surface with radial ribs crossed by numerpus, close-set concentric growth lines, their intersection with radial ribs making concellated aspect. Radial ribs about 27 in number, broad, low, flat, much broader than their narrow interspaces, broadest near middle, narrower and weaker and anterior and posterior extremeties. Concentric growth lines fine, closeset, appearing foliate or dorrugatc near ventral margin. Inner border coarsely crenulate. Hinge-line arcuate, with five teeth on anterior anterior and six on posterior parts, Area with four chrevons. A left valve. Length 16.0 mm., height 14.5 mm., depth 5.0 mm.

Remarks.—This species resembles *Glycymeris pectinata* (Lamarck) figured by S. Nomura and N. Zinbo (1934, Sci. Rep. Tohoku Imp. Univ., Ser. 2, vol. 16, no. 2, p. 112, pl. 5, figs. 1a, 1b) from the Pleistocene Ryukyu limestone of Kikai-jima, but can be distinuished therefrom by being longer than high, having fewer teeth on the anterior and posterior parts of the hinge-line, stronger and flatter radial ribs and a smaller shell.

Tucetona hanzawai (Nomura and Zinbo) from the same locality as *Glycymeris pectinaia*, is distinguished from the present fossil by having a higher shell and by the radial ribs being being nearly equal to their interspace, which is just opposite in the present species.

Station.-Shinkurose, St. 3. Lat. 33°24.5′N., Long. 140°10.0′E., 290 m.

BRACHIOPODA

Laqueus (?) sp.

A badly water worn fragment of the posterior half of the dorsal valve. The cardinalia, though badly worn, is apparently that of the named genus.

Statiop.-Shinkurose, St. 3. Let. 23°24 5′N., Long. 140°10.0′E., 290 m.

Thecidella japonica HAYASAKA

A single water worn valve of this rare species is represented in the collection. The type locality is stated by I. Hayasaka to be "off Hachijo Island".

Station.-Shinkurose, St. 3, Let. 33°24.5′N., Long. 140°10.0′E., 290 m.

MATERIAL OTHER THAN MOLLUSCA AND BRACHIOPODA

A very well preserved fish-scale was obtained from Shinkurose, Station 3 (Lat. 33°24.5′N., Long. 140°10.0′E.), together with several specimens of fish bone,

Two isolated branches of *Antipathes* sp., are represented in the collection from Shinkurose, Station 1 (Let. 33°30.0′N., Long. 140°07.5′E.. 280 m).

A fragment of a *Stylaster* colony is represented in the collection from Hachijo-jima, Station 1 (Lat. 33°08.5′N., Long. 139°45.0′E., 115 m).

Several brachioles with pinnules attached of some comaiulid crinoid are in the collection from Hachijo-jima, Station 1 (Let. 33°08.5N., Long. 139°45.0′ E., 115 m.). The specific names could not be determined owing to the unfavourable stated of the specimens.

Several broken tests and isolated spines of a cidaroid echinid are in the collection. The spines bear longitudinal striae and are reddish colored. The test is of a dark reddish color. These are from Hachijo-jima, Station 1 (Let. 33°08.5′N., Long. 139°45.0′E., 115 m.).

A beautiful reddish purple specimen, probably belonging to *Iodictyum sanguineum* (Ortmann) was found in the collection from Hachijo-jima, Station 2 (Lat. 33°08.5′N., Long. 139°45.0′E., 200 m.).

Mr. Yokochi Takeyanagi of the Institute of Geology and Paleontology, Tohoku University, kindly identified the following species of Foraminifera from Shinkurose, Station 3 (Lat. 33°30.0′N., Long. 140°10.0′E., 290 m.), namely:— *Dentalina* cf. *filiformis* (d,Orbigny), *Edonides* cf. *haidingeri* (d'Orbigny), *Globigerina inflata* d'Orbigny, *Globigerinoides conglobata* (Brady), *Globigerinoides rubra* (d' Orbigny), *Globigerinella aequilateralis* (Brady), *Globorotalia menardii* (d'Orbigny), *Guttulina eamazakii* Cushman & Ozawa, *Orbulina universa* d'Orbigny, *Pulleniatina obliquiloculata* (Parker & Jones), *Phyrgo depressa* (d'Orbigny, *Quinqueloculina contorta* d'Orbigny, *Robulus orbicularis* (d'Orbigny), *Textularia? transversalia* Brady, *Triloculina* cf. *tricarinata* d'Orbigny, and the following genera whose species have not yet been determined, *Biloculinella, Discopulvinulina, Eponides, Lingulina, Miliolinella, Robulus, Textularia,* and *Vaginulina.* The majority of these foraminifers are discolored and have the appearance of fossil rather than of Recent.